

THE ANNALS OF
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THE ANNALS OF APPLIED BIOLOGY

EDITED BY
W. B. BRIERLEY
AND
C. T. GIMINGHAM

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THE ANNALS OF APPLIED BIOLOGY

EDITED BY
J. B. S. HENDERSON
AND
H. D. H. HENDERSON

FOUNDED BY
J. B. S. HENDERSON

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February 1935

BY

AND

W. BROWN J. C. F. FRYER
A. D. COTTON H. F. BARNES
J. HENDERSON SMITH



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THE EFFECT ON THE TOMATO PLANT OF
CARBON DIOXIDE PRODUCED BY
COMBUSTION

By B. D. BOLAS AND R. MELVILLE.

(From the Research Institute of Plant Physiology, Imperial College of
Science and Technology, London, and the Experimental and Research
Station, Cheshunt, Herts.)

(With 7 Text-figures.)

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INTRODUCTION.

EARLIER work has shown that the enrichment of the atmosphere with carbon dioxide may increase both the vegetative growth and the fruit production of plants. In 1928 Bolas and Henderson(1) obtained substantial increases in the vegetative growth of cucumber and other plants under laboratory conditions, using carbon dioxide of high purity. Experiments in glasshouses at Cheshunt Experimental Station(2, 3), where carbon dioxide from several sources was tried, indicated a similar though less marked effect on the yield of tomato fruit.

The methods hitherto employed had been too expensive or too difficult of application for commercial purposes. With this in mind, one of us (B. D. B.), in 1928, experimented with the products of combustion of

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ordinary paraffin oil, burnt in a burner of the forced feed type. The increase in vegetative growth obtained under laboratory conditions with this device was smaller than with purer carbon dioxide, but was sufficiently marked to justify a trial on a larger scale in a small glasshouse at the Cheshunt Experimental Station. This experiment, carried out in 1929, gave an increase over the control of about 13 per cent. in the total yield of tomato fruit, in spite of indications of the entry of products other than carbon dioxide into the glasshouse.

In 1930 the work was repeated with slight changes in technique. A water spray was introduced into the system to wash the products of combustion before their entry into the glasshouse. At the same time, a more complete investigation was made of the distribution of carbon dioxide and its effects on the growth of the plants and the yield of fruit.

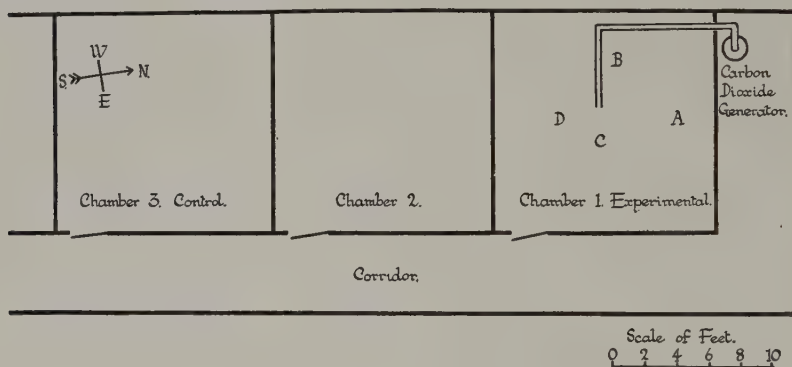


Fig. 1. Plan of the glasshouse.

THE GLASSHOUSE AND CARBON DIOXIDE GENERATOR.

The glasshouse used in these experiments was divided into six chambers arranged in a row and surrounded on three sides by a corridor. Each chamber was 14 ft. \times 14 ft. with sides 7 ft. high, and the height of the roof-ridge was 11 ft. Carbon dioxide was introduced into the first chamber at the north end of the house, while the third chamber was used for the control. Both were planted with 56 tomato plants of the variety E.S. 1 and were given the normal cultural treatment by the glasshouse staff.

The carbon dioxide generator was placed in the corridor outside chamber 1 (Fig. 1). Four "Primus" burners were employed, each capable of independent adjustment, but supplied with paraffin oil at a pressure of about 2 kg. per sq. cm. from a single pressure vessel. The

products of combustion were caught by a conical metal hood and let into the chamber through sheet iron piping of 10 cm. diameter. During their passage through the pipe they were washed by a water spray from a nozzle passing about 3 gallons of water per hour. Care was taken at all times to prevent the burners from smoking, and generally there was not the slightest smell of paraffin oil in the chamber.

THE MIXING OF CARBON DIOXIDE WITH AIR.

In order to determine whether a fan would be desirable for mixing the carbon dioxide with the air in the chamber, an examination of air currents was made; smoke from smouldering brown paper being used for this purpose.

It was found that air entering through the ventilators in the roof drove the warm gases downwards and set up a double circulatory movement (Fig. 2 A), which caused fairly complete mixing. This view was confirmed by determinations of the carbon dioxide content of samples of air taken from different parts of the chamber. It was concluded that no important advantage was to be gained by the use of a fan.

Further examinations of the air currents were made at intervals later in the season. When the tomato plants had reached a height of about 8 ft., the interference caused by the foliage resulted in the production of a single circulatory movement (Fig. 2 B). Occasionally, adverse wind conditions caused irregular currents.

In the control chamber and the remaining chambers in the glasshouse circulatory movements in the air were not found.

TEMPERATURE AND HUMIDITY.

Thermo-hygrographs were placed centrally in corresponding positions in the experimental and control chambers. The recorded differences in temperature and humidity were small, as indicated by the following mean values for the season:

		Experimental	Control
Mean temperature	Day	23.2° C.	23.3° C.
	Night	18.7° C.	18.1° C.
Mean relative humidity %	Day	63.2	65.2
	Night	78.2	82.9

Although there was little difference between the recorded temperatures, the possibility remained of considerable variations in different parts of the experimental chamber due to the entry of the warm gas stream. To investigate this point, a series of thermometer readings was taken in

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a median vertical plane at right angles to the direction of entry of the gas stream at 2 ft. intervals from ground level to the roof. The isotherms derived from these readings, plotted on a scale elevation of the chamber, are shown in Fig. 3. Weather conditions were uniform during the period midday to 2.30 p.m., in which the readings were taken, but the day was unusually hot. To this cause the general high level of temperature may

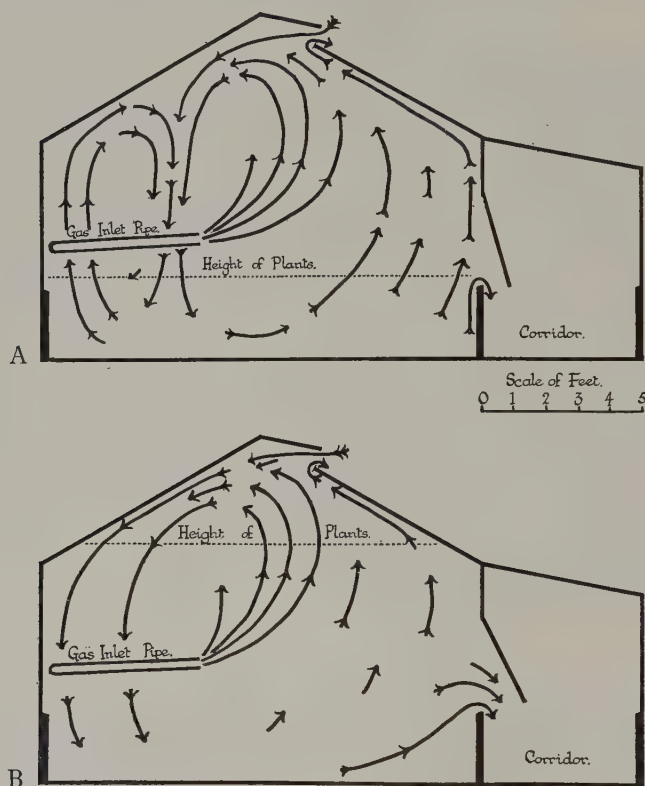


Fig. 2. Circulation of air in the experimental chamber.

be attributed. The shade temperature out of doors reached 33.9°C . and in the chambers the records gave:

Experimental	37.2°C .
Control	32.8°C .

Reference to the air circulation diagram, Fig. 2, will help to explain the form of the isotherms. The central dip in the 35°C . and 37.8°C . isotherms was due to the gas inlet pipe and the rising gas stream. The central break in the 39.4°C . isotherm may be accounted for by a down-

wardly directed stream of cool air from one of the roof ventilators. The discontinuity of the same isotherm towards the north wall suggests that from the second roof ventilator there was a downward air stream in that direction. The slight downward trend of the isotherms towards the north wall is in keeping with relatively high temperatures in the corridor outside this wall, where the paraffin burners were kept. The stratification of temperature observed was interfered with to a marked extent only where the gas stream entered. Elsewhere, the temperature conditions were satisfactory.

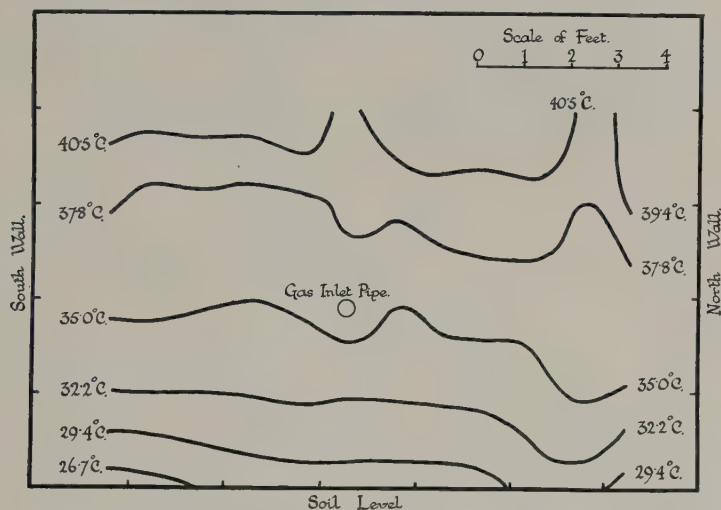


Fig. 3. Distribution of temperature in the experimental chamber.

AIR ANALYSES.

Samples of air were taken by displacement and analysed in a Haldane apparatus⁽⁴⁾ to determine their carbon dioxide content. Analyses of samples from different parts of the chamber (Table I) indicated a fairly uniform distribution of the gas.

Table I.

Distribution of carbon dioxide in the experimental chamber.

Date	Time	Height above soil	Position	CO ₂ , parts per 10,000
8. iv. 30	12.15 p.m.	1 ft. 6 in.	South-west corner	14.1
"	2.30	9 ft. 6 in.	Centre of south side	11.2
"	4.0	6 in.	Centre of south side	11.6
9. iv. 30	10.30 a.m.	1 ft. 6 in.	Centre of chamber	10.3
"	4.30 p.m.	5 ft. 0 in.	Centre of west wall	10.9

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As a result of the investigation of the air currents in the chamber, standard positions for taking air samples were adopted. These positions *A, B, C, D*, Fig. 1, were so chosen in relation to the air currents that large differences in carbon dioxide concentration might be expected. Except for the ascending gas stream, the variations in carbon dioxide content were, however, small. The means of a series of nine sets of estimations made between April 29th and May 9th are shown in Table II.

Table II.

Concentration of carbon dioxide in the standard positions.

Position	Height above soil	CO ₂ , parts per 10,000
<i>A.</i> Below the foliage	1 ft.	5.9
<i>B.</i> In descending air current	5 ft.	6.2
<i>C.</i> In ascending gas current	8 ft.	11.6
<i>D.</i> At top of foliage	3 ft.	5.4

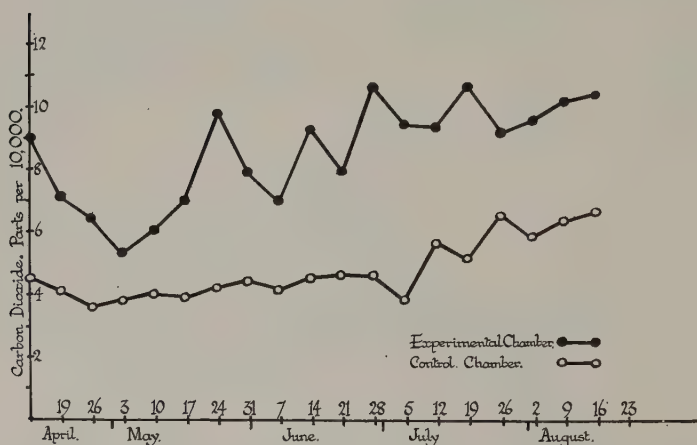


Fig. 4. Mean concentration of carbon dioxide in the experimental and control chambers for each week.

After these preliminary studies samples were taken whenever the generator was in use in both chamber 1 and the control chamber, at 4 ft. above soil level in position *D*. The weekly mean values for carbon dioxide concentration derived from these determinations are plotted in Fig. 4.

Though many factors contributed to cause the irregularities in the graph, it is possible to suggest some of the more important. From April 19th to May 10th, only three of the burners were in use. At the same time, vigorous growth was increasing leaf area and rising light intensity

helped to lower the carbon dioxide concentration. The cultural operations of "stopping" and removal of some of the older leaves on May 9th assisted the subsequent rise, though the high value of the mean for the week ending on May 24th was primarily due to an unusually high concentration, 22 parts per 10,000, on May 22nd. This was caused by closing the roof ventilators on account of the coldness of the day. The relatively high level of the graph for July and August may be correlated with the dull weather experienced.

CARBON DIOXIDE GRADIENTS.

The concentration of carbon dioxide in the control chamber was found to be slightly higher than that of the outside atmosphere and was observed to rise when the proportion in the experimental chamber was increased. This pointed to an appreciable leakage of gas along the corridor.

Analyses of air samples from corresponding positions in the experimental and the two adjacent chambers were made on fourteen days in May. The means of these in order were: 8·3, 4·6 and 4·2 parts of carbon dioxide per 10,000.

In view of this result, a more detailed investigation was undertaken. Samples of air from position *D* (Fig. 1) in all of the six chambers and from positions at regular intervals along the corridor were analysed.

The mean concentration of carbon dioxide found at each point is plotted in Fig. 5. A marked gradient of carbon dioxide existed in the corridor, where the concentration fell from 9·5 opposite the generator to 4·8 parts per 10,000 at the farther end. In the chambers, the gradient was less marked, though there was always a higher concentration than is usual in the atmosphere. It seems probable, therefore, that sufficient additional carbon dioxide was reaching the control chamber to have some effect on the crop.

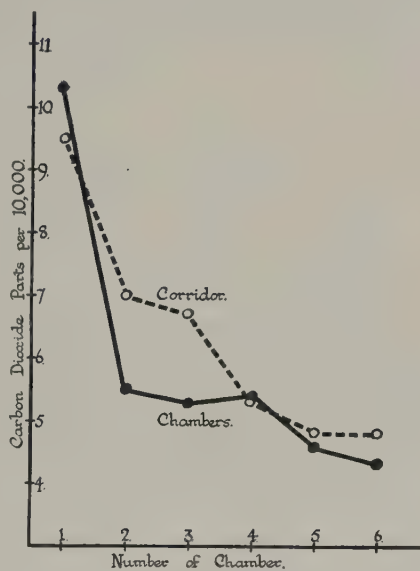


Fig. 5. Gradients of carbon dioxide in the chambers and the corridor of the glasshouse.

EFFICIENCY OF THE CARBON DIOXIDE GENERATING SYSTEM.

The presence of carbon dioxide gradients made it evident that losses of gas must be considerable, and it was of interest to determine the proportion actually entering the experimental chamber. The weight of gas entering was estimated from measurements of the carbon dioxide content of the gases issuing from the supply pipe, their speed and temperature and the diameter of the pipe. A Casella anemometer was used for obtaining the velocity of the gas stream.

The quantity of paraffin burnt per working day of six hours was about one gallon. If the hydrocarbon, decane, is taken as roughly equivalent to paraffin, the yield on complete combustion should be 11 kg. of carbon dioxide. The efficiency shown in Table III is the percentage of the latter figure of the weight of carbon dioxide found by experiment.

Table III.

Carbon dioxide entering the experimental chamber.

Date	Number of burners	Tempera- ture of gas °C.	Speed of gas m./min.	CO ₂ in gas parts in 10,000	Total CO ₂ in 6 hours gm.	Efficiency
11. iv. 30	3	76	70	138	4230	38.3
23. iv. 30	3	81	82	170	6023	54.7
21. viii. 30	4	75.5	91	208	8260	75.9

The estimation of April 11th indicated that less than half of the theoretical amount of carbon dioxide was entering the experimental chamber. Considerable loss of gas was found to take place at the edges of the metal hood above the burners. The losses at this point were much reduced by extending the hood to ground level for the greater part of its circumference and later estimations made after this alteration show an increase in efficiency. It is probable that the final figure, 75.9 per cent. of the theoretical value, was a fair measure of the efficiency of the apparatus under working conditions when four burners were in use.

To supplement the estimations of carbon dioxide entering the chamber, an attempt was made to measure the losses from the supply system. The more important sources of loss were likely to be from the edge of the hood covering the burners into the air in the corridor, in the wash water, and loss by incomplete combustion. No determination of the latter was made, though the lack of any smell in the chamber or of taste in the wash water suggests that loss from this cause was small.

On account of the frequent fluctuations in velocity and carbon dioxide content of the air streams arising from its orifice, it was difficult to make

a fair estimate of the loss from the hood. The values obtained are probably low. The loss shown was calculated from the means of a series of measurements of the carbon dioxide content and velocity of the air streams rising at a number of points along the aperture.

The volume of water used to wash the gas during six hours was 18 gallons. If this became saturated at its final temperature of about 40° C., it would contain 83.3 gm. of carbon dioxide, but the gases in contact with the water contained about 2 per cent. of carbon dioxide. If allowance is made for the partial pressure of carbon dioxide, the loss by solution would be of the order of 0.014 per cent. and therefore may be neglected.

A carbon dioxide balance sheet based on the above investigation is given below in Table IV.

Table IV.
Carbon dioxide balance sheet.

Carbon dioxide produced (calculated)...	11,000 gm.
„ passing into chamber	8,360 gm.			75.9 %		
„ lost round hood	351			3.2		
„ lost in wash water	2			0.014		
„ not accounted for	2,287			20.9		
Totals...	11,000			100.0		11,000

No quantitative study was made of the fate of the carbon dioxide which entered the chamber; sources of loss from the chamber may, however, be indicated. There was normally a drift of air outward from the chamber into the corridor at the corridor ventilator (Fig. 2 A), which contributed to the carbon dioxide gradients described. At the roof ventilator there was, in addition to the inflowing air stream, a narrow outward stream along its lower edge. There were probably smaller losses through crevices in the glass walls.

EFFECT OF THE TREATMENT ON THE PLANTS.

A number of differences were observed between the treated plants and the controls. Growth, judged both by the height of the plants and by their leaf areas, was stimulated. The mean heights at different growth-stages are shown in Table V. The increase may be accounted for by the production of longer internodes and a larger number of leaves.

Table V.
Mean height of plants.

Date	...	10. iv. 30	25. iv. 30	24. v. 30	13. vi. 30	23. vii. 30
Treated plants		2 ft.	3 ft. 6 in.	5 ft. 3 in.	6 ft.	8 ft. 6 in.
Control plants		1 ft. 9 in.	2 ft. 6 in.	4 ft. 3 in.	5 ft. 6 in.	7 ft. 6 in.

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A measurement of the total leaf area of the plants was considered impracticable, but on June 27th a comparison of the areas and dry weights of similar representative leaves was made. Ten leaflets from leaves immediately above the fifth truss of fruit were taken at random in both groups of plants. The areas were obtained by measuring tracings with a planimeter and the dry weights determined after 24 hours' drying in a water oven. The figures (Table VI) show that the carbon dioxide treatment had resulted in highly significant increases in area and dry weight. There was also some indication of an increase in the thickness of the leaves as shown by the dry weight per unit area, but this was not significant on account of the smallness of the samples.

Table VI.

	CO ₂ plants	Control plants	Difference	Increase %	<i>p</i>
Mean dry weight, mg.	231.3 ± 15.6	126.5 ± 7.4	104.8 ± 17.2	82.9 ± 13.6	0.01
Mean area, sq. cm.	63.8 ± 1.6	40.8 ± 2.1	23.0 ± 2.6	56.3 ± 6.3	0.01
Dry weight per unit area, mg./sq. cm.	3.59 ± 0.21	3.08 ± 0.01	0.51 ± 0.21	16.7 ± 6.8	0.2-0.1

"*p*" is the probability of the result being due to chance, estimated by the *t* test⁽⁵⁾.

No difference was observed in the susceptibility of the plants to infection by tomato mildew (*Cladosporium fulvum* Cke), which occurred in both groups of plants. Mosaic disease was also present and appeared to be slightly more severe in the treated plants.

The time of the onset of ripening of the fruit was the same in both, nor could any difference in the flavour of the fruit be detected. Counts of the number and condition of the trusses of fruit indicated that more fruit was set, especially in the early part of the season in the treated plants.

EFFECT OF THE TREATMENT ON THE CROP.

During the first half of the fruiting period an increase in crop of 29.3 per cent. was obtained from the treated plants, but for the second half the increase was 1 per cent. only. The very dull weather experienced during the latter half of July and most of August may account for the low increase during the second half of the crop period and the factor controlling fruit production was probably low light intensity rather than the carbon dioxide content of the atmosphere. A similar result was obtained by Small and White⁽³⁾ when experimenting with carbon dioxide from the combustion of a patent fuel during the wet and dull summer of 1927.

A possible effect of light is strikingly reflected in the two maxima of fruit production which occur in the middle of June and of August respec-

tively. These correspond with two periods of bright weather separated by a very dull interval. A more detailed examination of the weekly crop-records does not suggest a very close correlation of the ripening of the fruit with the sunshine records. Where a close correlation does occur, as in the case of the control crop at the end of August, the effect is probably due to the day temperature, which follows sunshine closely (Fig. 6).

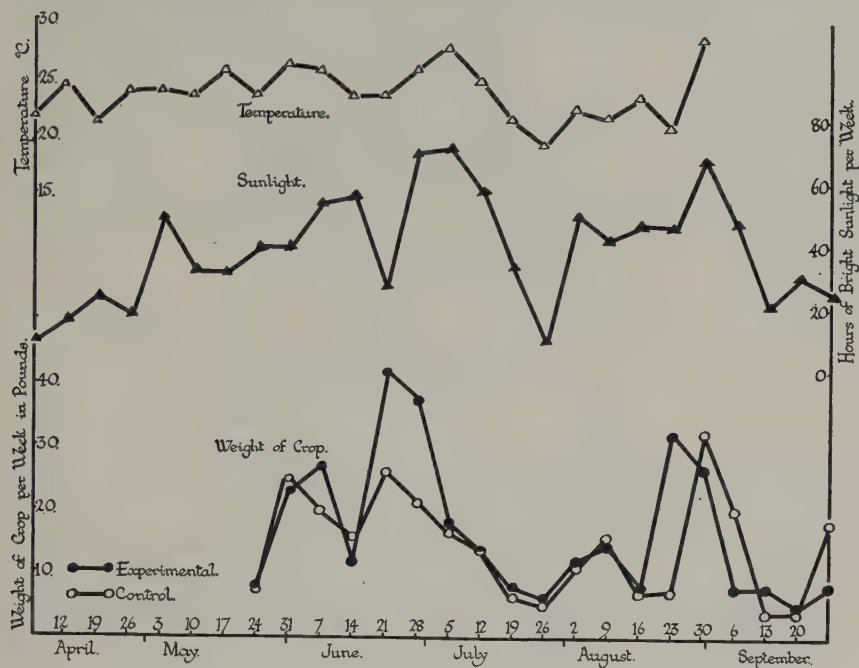


Fig. 6. Weight of crop each week, bright sunshine and temperature.

From the examination of sunshine records in relation to tomato crops of 1930 and other years, there would, however, appear to be some correlation between periods of bright weather occurring six to eight weeks before the gathering of the fruit, during its early development.

A summary of the crop records is given in Table VII and the weekly fruit pickings are plotted in Fig. 6.

Table VII.

Summary of crop records.

	First half, May 24-July 26	Second half, July 27-Sept. 27	Total for season
Weight of experimental crop	195.3 lb.	122.4 lb.	317.7 lb.
Weight of control crop	157.8 lb.	121.2 lb.	279.0 lb.
Increase in yield	29.3 %	1.0 %	13.9 %

Table VIII.
Dry weight of seedlings.

No. of exp.	Date of start	Duration days	No. of days' gas	Carbon dioxide plants				Control plants				<i>p</i>
				Initial dry wt. mg.	No. in sample	Final dry wt. mg.	No. in sample	Initial dry wt. mg.	No. in sample	Final dry wt. mg.	No. in sample	
1 <i>a</i>	12. v. 30	8	5	5.0 ± 0.1	20	48.0 ± 1.8	10	5.0 ± 0.1	20	50.9 ± 1.4	10	0.4
1 <i>b</i>	"	14	10	5.0 ± 0.1	20	298.0 ± 10.7	12	5.0 ± 0.1	20	265.0 ± 8.7	12	0.2-0.1
2	11. vi. 30	12	8	3.0 ± 0.003	30	121.0 ± 1.8	30	3.0 ± 0.003	30	93.0 ± 2.3	26	0.01
3 <i>a</i>	23. vi. 30	7	6	12.7 ± 0.2	40	91.0 ± 2.7	29	12.4 ± 0.2	40	86.5 ± 1.8	34	0.4-0.3
3 <i>b</i>	"	14	12	12.7 ± 0.2	40	525.5 ± 9.7	40	12.4 ± 0.2	40	436.3 ± 7.2	40	0.01
4 <i>a</i>	8. vii. 30	3	3	23.1 ± 0.5	30	60.9 ± 1.3	30	25.9 ± 0.6	30	57.6 ± 1.1	30	0.4-0.3
4 <i>b</i>	"	9	7	23.1 ± 0.5	30	311.5 ± 5.1	30	25.9 ± 0.6	30	308.5 ± 6.0	30	0.9-0.8
5	21. vii. 30	16	6	252.3 ± 4.5	40	521.2 ± 5.4	17	252.3 ± 4.5	40	501.7 ± 4.3	20	0.01

EXPERIMENTS ON THE GROWTH RATE OF TOMATO SEEDLINGS.

Determinations of the growth rate of seedlings of the same variety as the crop plants (E.S. 1) were made at intervals during the season by the dry weight method. Small seedlings in seed trays were used in all but the last experiment for which larger seedlings in pots were employed. As far as possible, conditions of growth were similar for both groups of plants apart from the addition of carbon dioxide. In Exps. 1 and 4 the result was biased against the experimental plants by slight shading from the crop plants in chamber 1, while the controls remained unshaded.

The plants were sampled by cutting at soil level at the commencement and once or twice during each experiment. Each plant was weighed separately after 24 hours' drying in a water oven. Details of the experiments are given in Table VIII, in which p is obtained by "Student's" method.

The mean growth rates K of the seedlings were calculated from the results, using the formula (6)

$$K = \frac{\log_{10} Q_t - \log_{10} Q_0}{t \log_{10} e},$$

and are shown in Table IX. In seven out of the eight experiments, the treatment resulted in an increase in the growth rate, with a mean increase of 5.37 per cent. for the series. The probability of this result being due to chance, determined by the " t " test, lies between 0.1 and 0.05. This probability may be halved since a unidirectional change was expected, and the result of the series of growth experiments is therefore significant. But for the adverse bias due to dull weather, escape of carbon dioxide and slight shading, the significance would probably have been much higher.

Table IX.

Expt. No.	Growth rate of carbon dioxide plants	Growth rate of control plants	Per cent. increase in growth rate
1 <i>a</i>	0.2827	0.2859	-1.12
1 <i>b</i>	0.2919	0.2834	3.00
2	0.3080	0.2865	7.50
3 <i>a</i>	0.2822	0.2781	1.47
3 <i>b</i>	0.2659	0.2543	4.56
4 <i>a</i>	0.3234	0.2663	21.44
4 <i>b</i>	0.2892	0.2754	5.01
5	0.1895	0.1875	1.07
Mean increase in growth rate			5.37

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COST OF THE TREATMENT AND ECONOMIC BEARING OF THE RESULTS

The carbon dioxide generator was used on 86 days during the season and a total of 92.5 gallons of paraffin oil was burnt. The cost of this was £5. 4s. 1½d. and the cost of 1.075 gallons used per working day was 14.5 pence.

The treatment resulted in an increase of crop of 38.8 pounds at a cost per pound of 32.2 pence for paraffin. This figure is plainly uneconomic.

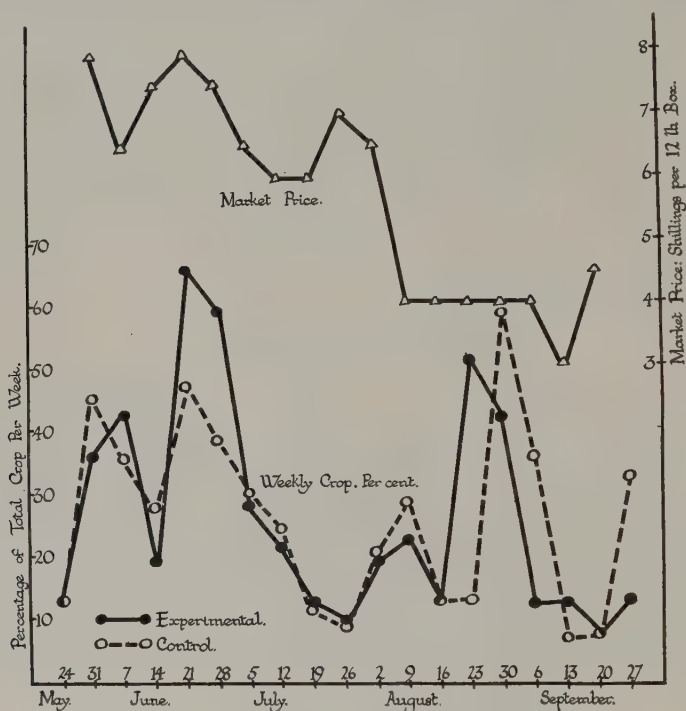


Fig. 7. Proportion of crop each week and market prices.

It must be pointed out, however, that in working on a larger scale it should be possible to reduce the amount of paraffin required on account of the smaller losses to be expected from a large glasshouse. With more efficient apparatus much of the loss of gas experienced in the present experiment could be avoided. It is probable, also, that when data are available as to the conditions of light and temperature under which the tomato plant is able to take advantage of additional carbon dioxide, that still further reductions may be made in the cost of the treatment. In the present work, gas was withheld only on very dull days.

The proportion of the crop gathered each week and the current market prices of tomatoes were compared (Fig. 7). The major portion of the increase in the crop was obtained early in the season at the time when prices were high. Other investigators (2, 3) have obtained similar results. The economic bearing of this is obvious. Later in the season, more than double the increase in crop would have to be produced to give the same return. From these considerations it seems probable that carbon dioxide culture for the tomato is likely to be of commercial value only in the first half of the season.

SUMMARY.

The effect of carbon dioxide on tomato plants growing in a small greenhouse has been studied, the gas being produced by burning paraffin in a pressure burner.

An increased yield of fruit of 23.9 per cent. for the first half of the season and 13.9 per cent. for the whole season was obtained.

A significant increase in the growth-rate of tomato seedlings was brought about by the treatment.

The distribution of carbon dioxide in the glasshouse was investigated and the sources of loss of gas examined.

The economic bearing of the results is briefly discussed.

The authors wish to acknowledge their indebtedness to Prof. V. H. Blackman, F.R.S., for his unfailing interest and helpful suggestions during the course of the work, and to Dr W. F. Bewley, Director of the Experimental and Research Station, Cheshunt, for providing accommodation and materials.

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PREVENTION OF BLIGHT (*PHYTOPHTHORA INFESTANS*) IN SEED POTATOES

BY T. SMALL, PH.D., A.R.C.S.

(*States Experimental Station, Jersey, C.I.*)

IN Jersey, potato blight (*Phytophthora infestans* (Mont.) de Bary) causes serious loss in seed potatoes every year, amounting in many cases to more than half the crop. Occasionally it results in a shortage of the favourite variety, *International Kidney*, and other less desirable varieties have to be imported to complete the plantings. In view of the probability of disease reducing their stocks, farmers usually reserve seed in excess of their requirements; this means that less produce is available for export to England.

As will be shown later, much of the damage is caused by digging the seed when blight is prevalent on the green haulms. This is often unavoidable as harvesting cannot be delayed until the haulms have died down, because it is carried out by labour imported for a limited period, and because, owing to the high value of land, the ground must be cleared to make room for valuable second crops such as tomatoes, broccoli and roots. In addition, experience and experiments indicate that early dug seed yields earlier crops the following season.

The loss may be subdivided into: (a) Loss in the field. This includes diseased tubers left on the ground at harvesting time. (b) Loss in the seed boxes. As the crop is dug, apparently sound seed is placed in boxes and stored at the farm. After a few weeks the tubers are examined for blight.

SOURCE OF INFECTION.

This was determined by digging tubers from crops in various stages of disease. The loss in the field was noted and the apparently healthy seed was transferred to boxes, placed in a moist chamber and examined later. The results were as follows:

(a) *Healthy crops.* No diseased tubers at digging time or subsequently.

(b) *Recent attack.* Here the haulms are still green but disease is prevalent on them and the fungus is sporulating freely. Harvesting at this stage gave little or no loss in the field but a serious one in the boxes, as shown in Table I.

(c) *Old attack.* The haulms have been killed by blight and are brown, dry and shrivelled; few, if any, spores are present. The loss at digging time was usually serious and that subsequently was negligible.

(d) *Attack intermediate between (b) and (c).* In these cases some loss occurred in the field and also in the boxes.

Table I.

Development of blight in seed tubers from diseased crops.

Date dug	Date examined	Condition of tubers		
		No. healthy	No. diseased	Diseased %
29. vi. 32	13. vii. 32	20	180	90
31. vi. 32	14. vi. 32	22	108	83
26. vi. 33	3. vii. 33	47	147	76
24. vi. 32	30. vi. 32	80	114	59
26. vi. 33	3. vii. 33	55	65	54
14. vi. 32	29. vi. 32	91	48	34
7. vi. 32	22. vi. 32	190	56	23

The results show that the number of diseased tubers at harvesting time and in the boxes was related to the age of the attack on the haulms. The loss in the field was serious only in the case of older attacks whilst that in the boxes was severe only in the case of recent attacks. In the latter case spores were plentiful on the haulms and it is very probable, as shown by Murphy and McKay⁽¹⁾, that these fell on the tubers at digging time and so caused disease later in the boxes. Experiments showed that diseased haulms shaken over healthy tubers resulted in the latter developing blight. Few, if any, spores were present on the haulms in the case of old attacks and seed which was healthy at harvesting remained healthy in the boxes.

The results suggested that the loss in the field could be avoided by preventing the disease from reaching an advanced stage before harvesting time, and that most of the disease in the boxes could be checked either by removing the source of infection before digging or by treating the tubers so as to kill the spores which fall on them at harvesting time.

PREVENTION OF LOSS IN THE FIELD.

Numerous spraying trials were made from 1931 to 1934 in many parts of the Island. The spray was neutral to litmus and consisted of 4 lb. copper sulphate and $1\frac{1}{8}$ lb. of caustic soda in 40 gallons of water. It was applied at fortnightly intervals from early May (when the plants were 6-8 in. high) to late June, using a knapsack sprayer or a horse-drawn machine. The crops were harvested in late June or early July. The results

showed conclusively that regular and thorough spraying delayed the onset and spread of blight sufficiently to eliminate loss in the field. In the case of unsprayed control plots losses up to 20 per cent. of the crop were experienced except in 1934 when all the plots remained healthy. It is concluded that spraying, properly carried out, will prevent this loss in most seasons.

PREVENTION OF LOSS IN SEED BOXES.

While spraying may prevent loss at harvesting time, it cannot always be relied upon to check disease in the boxes. In some seasons and in some localities sprayed crops remained sound and no further precaution was necessary to keep the seed healthy. In other trials, however, disease appeared on sprayed plots and caused much loss in the boxes (see Table I). Many growers have experienced this and have abandoned spraying. They prefer to leave the seed crop unsprayed and risk the loss in the field. This method cannot be recommended for many reasons. It is far too risky because blight may appear early in the season and destroy the crop; diseased crops are a source of danger to neighbouring crops, and lastly, as is shown later, it is possible to prevent loss in the boxes.

Four methods were investigated to keep the seed healthy in cases where, in spite of spraying, the haulms were attacked by blight near lifting time.

A. Before harvesting:

- (1) Scorching the haulms.
- (2) Removing the haulms and spraying the soil.

B. After harvesting:

- (3) Leaving the seed outside in boxes.
- (4) Immersing the seed in fungicides.

(1) SCORCHING THE HAULMS.

This method aims at killing the disease on the haulms by using an acid spray. Various sprays were tested, with and without spreaders, and a mixture of 12 lb. copper sulphate and $\frac{1}{4}$ lb. caustic soda in 40 gallons of water proved effective, especially in sunny weather. The best results were obtained where two heavy applications were given, allowing a three-day interval, and the crop was harvested a few days later. In addition to killing most of the spores, the scorching of the haulms allows the sun to dry the soil, thus reducing further the risk of disease in the tubers. The method has been tried on a field scale and careful growers have obtained satisfactory results.

(2) REMOVING THE HAULMS AND SPRAYING THE SOIL.

In these experiments the diseased haulms were cut with a scythe and removed, after which the ground was sprayed and the seed harvested. The results of one trial are given in Table II, and show that much disease occurred where the haulms were not cut and also where they were cut and the crop dug immediately. Least disease developed where the haulms were cut and the crop dug three days later. It should be noted here that the haulms were cut in sunny weather and that this weather continued until the crop was dug. There is evidence to show that less favourable results might be obtained in rainy weather. Spraying the ground reduced the amount of disease but not sufficiently, and the experiments are being continued. The best results are likely to be obtained where the haulms are cut and removed in dry weather, the ground sprayed at once and the crop dug three days later.

Table II.

Development of blight in seed tubers after removal of haulms.

Condition of haulms	Treatment	Loss in seed boxes		
		No. of diseased tubers	No. of boxes	Average per box
Disease prevalent on partly green and partly dead foliage	Haulms cut; crop dug 3 days later	148	22	7
" " "	Haulms cut; ground sprayed and crop dug at once	147	15	10
" " "	Haulms not cut	378	23	16
" " "	Haulms cut; crop dug at once	441	20	22

2 gallons of Eau Celeste (1 in 80) per 54 sq. yd.

(3) LEAVING THE SEED OUTSIDE IN BOXES.

It is a common practice among growers to leave seed outside in boxes for about one week after digging, the object being to "green" and harden the tubers and so prevent disease.

The method was tested by inoculating newly dug, healthy seed with a spore suspension from fresh diseased leaves, placing it outside in boxes for different periods, and then transferring it to a moist chamber. The results of one experiment are given in Table III and show that although there was less disease in the boxes left outside, the loss was considerable in all cases. Similar results were obtained in further experiments and it is

concluded that the method cannot be recommended since much loss may occur especially in wet weather.

Table III.

Development of blight of seed tubers left outside in boxes.

No. of days tubers left outside after inoculation	Weather conditions* Rainfall	Tubers		
		No. healthy	No. diseased	Diseased %
0	—	14	84	86
3	Nil	63	24	28
5	0.04 in. on 3rd night	74	21	22
7	0.04 in. „	—	—	—
	1.04 in. on 6th night and day	59	25	30

* The maximum temperature was 24° C. and the minimum temperature 10° C.

(4) IMMERSING THE SEED IN FUNGICIDES.

This method aims at killing the spores which fall on the seed at harvesting time. Preliminary trials were made in the laboratory. Freshly dug seed was inoculated with a spore suspension and allowed to dry. It was then placed in boxes, momentarily immersed twice in the fungicide and afterwards placed in a moist chamber. When formaldehyde was used the vapours were allowed to pass off before placing the seed in the chamber. Inoculated controls remained undipped or were dipped in water. The results are given in Table IV and show that the treatment prevented blight.

Table IV.

Immersion of inoculated tubers in fungicides.

Date of inoculation	Treatment	Tubers		
		No. healthy	No. diseased	Diseased %
7. vi. 32	Immersed twice in formaldehyde (1 %)*	300	1	—
„	„ „ water (control)	69	302	82
27. vi. 32	„ „ boric acid (3 %)	193	2	1
„	„ „ copper sulphate (1 %)	169	2	1
„	„ „ water (control)	64	41	39
„	Not immersed (control)	63	42	40
15. vi. 32	Immersed twice in spray mixture†	435	9	6
„	„ „ water (control)	106	362	77

* 1 pint 40 % formaldehyde in 99 pints of water.

† See p. 17.

The method was then tried under field conditions and for this purpose apparently healthy tubers from diseased crops were dipped immediately after digging. The results are given in Tables V and VI and show that the

treatment was effective except in one experiment (No. 3 in Table VI). It is to be expected that some disease may occur occasionally in spite of the dipping, because a few tubers are probably infected, but not visibly, when dug. In older attacks the number of such infected tubers may be appreciable. The maximum benefit of the dipping will be secured where the attack on the haulms is prevalent but recent.

Table V.

Immersion of tubers from diseased crops in fungicides.

Treatment	Tubers		
	No. healthy	No. diseased	Diseased %
Immersed twice in formaldehyde (1 %)*	256	2	1
" " water (control)	190	56	23
" " spray mixture†	115	2	2
" " water (control)	91	48	35

* 1 pint of 40 % formaldehyde in 99 pints of water.

† See p. 17.

Table VI.

Immersion of tubers from diseased crops in formaldehyde (1 per cent.).

Treatment	No. of tubers	
	which developed blight	Av. per box
Expt 1 { 12 boxes dipped	56	5
{ 12 boxes not dipped	97	8
2 { 25 boxes dipped	51	2
{ 15 boxes not dipped	213	14
3 { 18 boxes dipped	175	10
{ 16 boxes not dipped	281	17

The treatment is simple, practical and inexpensive. The apparatus consists of:

(1) A low, wide wooden tub containing sufficient fungicide to allow complete immersion of the seed box full of tubers.

(2) A piece of corrugated iron or other support to form a draining board.

(3) A wooden container with high sides and two handles and just large enough to hold a seed box. It prevents the tubers falling from the seed box during immersion.

(4) The fungicide recommended is a 1 per cent. dilution of formaldehyde (1 pint of 40 per cent. in 99 pints of water). Fifteen gallons are sufficient for hundreds of boxes.

The seed is placed in the boxes when dug in the field and brought to the farm. As each box is unloaded it is placed in the wooden container,

dipped in the fungicide, and transferred to the draining board to allow excess fungicide to run back into the tub. The box is then stacked in the usual way. With four men working, one unloading the boxes, two dipping and one stacking, 360 boxes of seed were treated in 1 hour. The formaldehyde is not injurious; dilutions up to 3 per cent. have been tested and the treated and untreated seed sprouted equally well. It is obvious, of course, that the seed must be dipped soon after digging, before the spores have germinated and infected the tubers.

SUMMARY.

1. The loss of seed potatoes through blight (*Phytophthora infestans*) in Jersey may be subdivided into (a) loss in the field at digging time, and (b) loss in the seed boxes subsequent to harvesting.

2. It is shown that the loss in the field may be prevented in most seasons by regular and thorough spraying, whilst that in the boxes may be reduced by scorching or removing the diseased haulms before digging, or by immersing the tubers in a fungicide.

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ON THE OCCURRENCE OF *APLANOBACTER*
RATHAYI E. F. SMITH ON *DACTYLIS*
GLOMERATA IN ENGLAND

BY W. J. DOWSON AND M. d'OLIVEIRA.

(*Botany School, Cambridge.*)

(With Plate I.)

THE bacterial disease of cocksfoot grass, *Dactylis glomerata*, has long been known on the Continent, where it was first investigated by the late Prof. Rathay at Vienna in 1897⁽¹⁾, and has been constantly reported since then, particularly from Denmark⁽²⁾. In 1914 E. F. Smith⁽³⁾ gave a summary of Rathay's work and his own studies of the organism concerned, which he named *Aplanobacter Rathayi*. C. Elliot⁽⁴⁾ records the literature on the disease up to 1928 and von Oettingen⁽⁵⁾ reported severe damage to this grass in Germany during the summer of 1932.

That the disease can be destructive at times is evident from the quotations given by Elliot and from the more recent account by von Oettingen. Its occurrence in England may therefore be of some importance, although it seems probable that the disease has been here for some time unrecognised. Mr W. C. Moore of the Ministry of Agriculture, Plant Pathological Laboratory, Harpenden, informs us that Miss K. Sampson at Aberystwyth received a specimen of cocksfoot in May 1932 which she believed to be attacked by *A. Rathayi*. Furthermore, the Official Seed Testing Station for England and Wales, Cambridge, has informed us that the presence of a dried yellow exudate on cocksfoot seed has been noted on several occasions in samples of English grown seed but that imported Danish seed is more commonly affected. We have examined some of these samples and find that a viable organism is present corresponding in its characters to *A. Rathayi*.

On May 27th, 1934, one of us (M. d'O.) found about 100 diseased plants of *D. glomerata* at Clayhithe, near Cambridge, scattered over an area of about 200 sq. yd., extending along the left bank of the Cam between the river and tow-path. Two days later a single plant similarly affected was found near the edge of a pasture close to the University Farm, Cambridge. Both situations were open, well drained and in no way shaded, whereas the affected plants found near Vienna were growing

in woodland. The infected plant found near the University Farm was carefully dug up, potted and kept on the roof of the Botany School, Cambridge.

The general appearance of the affected plants closely resembled the descriptions and illustrations of the disease given by Smith, the most general symptom being the presence of yellow droplets of bacterial slime at the base of the spikelets, the lower parts of the flowering stems being straight and clean. Only a few plants were badly infected, having the leaves and stems stuck together by copious yellow slime, which prevented the emergence of the inflorescence; at a later stage the flowering shoots of such plants were wholly destroyed, leaving a dried-up brown residue. In other inflorescences the upper parts were covered with the slime and much contorted (Plate I, figs. 1, 2), as depicted by Smith. In no instance were the basal parts diseased nor was any stunting noticeable; and the plants were tillering in a normal way. These symptoms correspond with the original descriptions of the disease but not with the latest account given by von Oettingen, who records killed-out patches of *Dactylis*, severe stunting and little or no tillering.

A watery suspension of the yellow slime showed predominantly a non-motile, capsulated, rod-shaped bacterium, which stained by Gram's method. Poured plates of potato glucose agar, inoculated with the slime, gave rise to small, bright yellow colonies of Gram-positive organisms in from 60 to 72 hours, mixed with which were a few yellow colonies of Gram-negative bacteria and also white colonies of Gram-positive bacteria. The first-mentioned organism was selected for further study and was found to agree with the descriptions and cultural characters of *A. Rathayi* E. F. Smith. The only addition we have to make is that the organism will not grow in Fermi's or Uschinsky's solution.

We have found, just as Smith did, that the organism grows very slowly on culture media when first isolated or when transferred at long intervals. After subculturing every five or six days on potato glucose agar a faster and more abundant growth was obtained. Steamed potato seemed to be the best medium, and on this the growth is viscid and bright yellow, smooth so long as the substratum keeps moist, becoming wrinkled when dry. In potato extract, with or without glucose, growth is abundant with the formation of a precipitate.

In confirmation of Rathay's investigations we have been unable to produce the disease in healthy plants inoculated with pure cultures. On the other hand, the yellow slime, as it occurs on naturally infected plants, proved to be pathogenic when introduced into the shoot of a healthy

plant. Thus by means of a scalpel the yellow slime was introduced between the leaf sheaths at the base of two young vigorously growing shoots. After about a fortnight one of the inoculated shoots appeared slightly swollen at the base. The swelling increased in size and a week later an exudate of yellow slime appeared on the outside of the leaves. The swelling then split and through the opening the much contorted stem protruded covered with slime (Plate I, fig. 3). The other inoculated shoot was not infected and, after a period of drought with some days of intense heat, heavy rain fell and the plant died, becoming a wet rotten mass. A shoot on a second plant inoculated one week after the first had just commenced to swell at the point of inoculation when the change in weather occurred. The infection was then checked, for the plant recovered shortly afterwards.

SUMMARY.

1. The occurrence of bacteriosis of *D. glomerata*, with which is associated the non-motile organism *A. Rathayi* E. F. Smith, is recorded for Cambridgeshire definitely for the first time, but there seems little doubt that the disease has been present unrecognised for some time and is being constantly introduced on imported Danish seed.

2. The disease is similar to that originally observed near Vienna in 1897, but differs from the latest account from Germany where the damage caused was far more severe. The general symptoms were partial or complete destruction of the spikelets which were embedded in a bright yellow bacterial slime.

3. The associated organism was isolated and found to agree exactly in its cultural and growth characters with the descriptions of Rathay and Smith.

4. The naturally occurring bacterial slime proved pathogenic when inoculated into a healthy shoot of *Dactylis*, but no infection resulted when pure cultures on artificial media were used.

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EXPLANATION OF PLATE I.

- Fig. 1. Inflorescence of *D. glomerata* infected by *A. Rathayi*, showing distortion of the axis and destruction of the basal spikelets. All the black part represents yellow bacterial slime.
- Fig. 2. Four heads of *D. glomerata* showing different degrees of infection: *A.* Complete destruction of spikelets. *B.* Poor development of inflorescence and spikelets stuck together with bacterial slime (black in illustration). *C.* Zig-zag contortion of the axis well marked. *D.* Basal spikelets only infected and covered with yellow slime (black in illustration).
- Fig. 3. Plant inoculated with yellow slime from a naturally infected plant, showing basal swelling of the shoot and the inner leaves protruding through a split of the outer leaves which are stuck together with slime.

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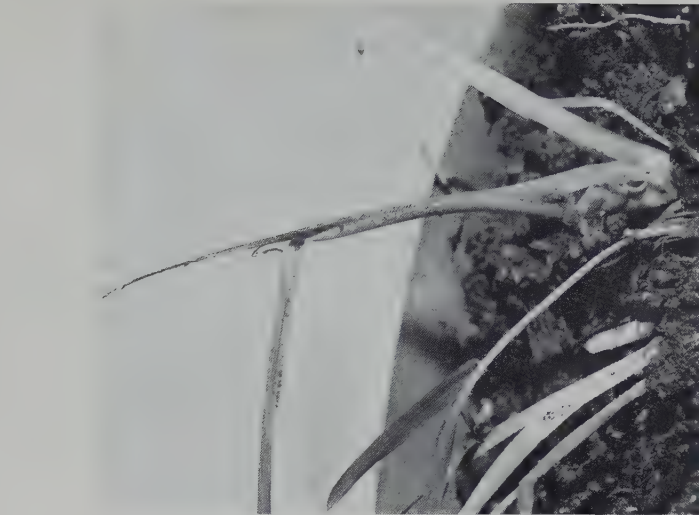


Fig. 3.



D

C

B

A

Fig. 2.

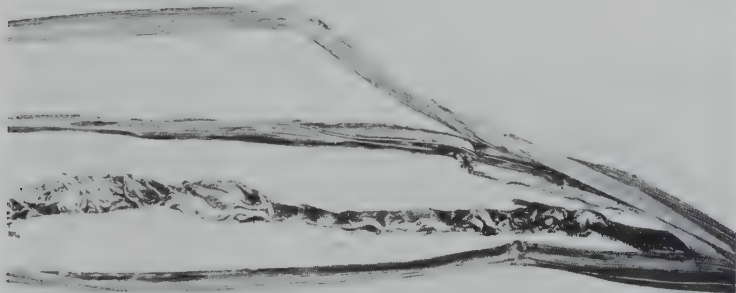


Fig. 1.

TWO VIRUSES OF THE CUCUMBER MOSAIC GROUP ON TOBACCO

BY ISMÉ A. HOGGAN.

(*Wisconsin Agricultural Experiment Station in co-operation with the Division of Tobacco and Plant Nutrition, United States Department of Agriculture.*)

(With Plate II.)

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INTRODUCTION.

THE recent conception of the existence of a number of distinct plant viruses with fairly stable characteristics is now being modified to meet the rapidly accumulating evidence of the occurrence of strains or related forms of these viruses. Such related strains are usually distinguishable either by the symptoms produced, while other, more fundamental characters remain identical, or by a differential host range, in which case the symptoms on a common host may be the same. Thus, Storey and McClean(15) have described a number of distinct forms of streak virus in the Gramineae, varying in intensity of symptoms on a particular host, but all transmissible by the same insect vector, *Cicadulina mbila* N. Similarly, Kunkel's comparison of the New York and California aster yellows(11), which differ in their ability to infect celery, leads one to suspect that two distinct strains of one virus are here concerned. Other examples of this type are known, including strain variation in the ordinary tobacco mosaic virus (5, 8, 12, 14), the potato veinbanding virus(9), and so on.

Evidence has been accumulating also of strain variation in the ordinary cucumber mosaic virus (*cucumber virus 1*)(7). Jagger(4), Bewley(1),

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Porter⁽¹³⁾ and E. M. Johnson⁽⁶⁾ have distinguished, mainly on the basis of symptoms, various types of cucumber mosaic, some at least of which appear to be due to different strains of this virus, though the relation is not always clear from the descriptions given. The more recent work of Doolittle and Wellman⁽²⁾ on a celery mosaic virus occurring in Florida has shown that this form is also in many respects very close to the ordinary cucumber mosaic virus and is perhaps best regarded as a strain of the latter. In the present paper are described more fully two viruses, one of which, a "yellow cucumber mosaic virus¹," is undoubtedly a strain variant of the ordinary form, and the second, although showing differences of greater magnitude, is also regarded as belonging to the same group.

THE "YELLOW CUCUMBER MOSAIC VIRUS."

This virus appeared to develop spontaneously on two separate occasions during greenhouse trials of aphid transmission of the ordinary cucumber mosaic virus on tobacco and related hosts, some phases of which have been reported earlier⁽³⁾. On the first occasion (June 8th, 1928), about twenty-five individuals of *Myzus persicae* Sulz. were transferred by the usual method from cucumber-mosaic-diseased tobacco to each of four healthy tobacco plants. Six days later, two of these plants developed symptoms of ordinary cucumber mosaic, one remained healthy, and the fourth developed a distinct form of mosaic in which the lighter areas of the leaf were bright lemon-yellow in colour in place of the normal pale green characteristic of cucumber mosaic on this host. The four control plants remained healthy. Needle inoculation to tobacco with extract from this yellow-mosaic plant yielded both yellow mosaic and ordinary cucumber mosaic on different plants. Evidently there was some unchanged cucumber virus associated with the yellow form. In subsequent transfers, however, a strain was recovered that consistently produced a marked yellow mosaic on tobacco and with which no ordinary cucumber mosaic virus appeared to be associated. This yellow form was maintained unchanged on tobacco for nearly five years, when it was finally discarded.

On the second occasion (April 12th, 1933), several months after all material of the original yellow mosaic had been discarded from the greenhouse, three of four tobacco plants, to which aphids (*M. persicae*) had been transferred a week earlier from cucumber-mosaic-diseased tomato, developed symptoms of ordinary cucumber mosaic, while the fourth

¹ After this manuscript was prepared, a paper by W. C. Price appeared containing descriptions of several strains of yellow cucumber mosaic. (Price, W. C. (1934). Isolation and study of some yellow strains of cucumber mosaic. *Phytopathology*, xxiv, 743-61.)

plant developed a form of yellow mosaic closely resembling in symptoms the yellow mosaic previously obtained. All control plants again remained healthy. The circumstances in both cases pointed to the yellow form having developed in some way from the original cucumber mosaic virus used in the tests, though whether as a sudden "mutation" or in some other manner was not clear. That the virus represented an accidental contaminant introduced from some other source seemed unlikely, for, apart from the regular precautions observed throughout in this kind of greenhouse work, it was too early, at least on the second occasion, for aphids or other sucking insects to be active out-of-doors, nor was any yellow mosaic of the type in question present elsewhere in the greenhouse or in adjacent houses at the time of its development in these trials.

First symptoms of the yellow mosaic on tobacco usually consisted of small, bright yellow spots on the young leaves, generally close to the veins. These spots enlarged rapidly until large areas of the leaf were involved, becoming pale yellow or sometimes almost white in colour (Plate II, fig. 1 *A, B*). Under certain conditions, local lesions, in the form of round yellow spots, developed on leaves inoculated by rubbing, usually a short time before first signs of systemic infection. While the symptom pattern, apart from the yellow discoloration, resembled fairly closely that of ordinary cucumber mosaic, the yellow form was somewhat more severe in its effects, causing more decided stunting of the plant and a gradual necrosis of the lower leaves, besides showing, under certain conditions, a slightly shorter incubation period.

Further studies of the yellow mosaic virus have shown it to be identical in modes of transmission, properties and host range, so far as studied, with the ordinary cucumber mosaic virus, and it is therefore designated as the "yellow cucumber mosaic virus." Thus, the yellow cucumber mosaic virus proved to be readily transmitted by aphids (*M. persicae*) as well as by plant extract (needle and rubbing inoculation). In a comparison of properties of the two viruses in tobacco plant extract, the thermal death-point in each case was found to be 71° C. for a 10 min. treatment (Table I), the tolerance to dilution 1 in 100,000 (Table II), and the longevity *in vitro*, at about 21° C., between 5 and 6 days for the yellow form and between 5 and 7 days for the ordinary form (Table III). The yellow cucumber mosaic virus was readily transmitted to cucumber (*Cucumis sativus* L.), tomato (*Lycopersicon esculentum* Mill.), pokeweed (*Phytolacca decandra* L.), spinach (*Spinacia oleracea* L. var Bloomsdale), nightshade (*Solanum nigrum* L.) and egg-plant (*S. melongena* L.), all of which species are susceptible to ordinary cucumber mosaic. On these hosts, in addition to the

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distinctive yellow discoloration, the yellow cucumber mosaic virus caused more severe necrosis of the foliage and slightly more marked stunting of the plant than the ordinary cucumber mosaic virus, but somewhat less

Table I.

Comparison of thermal death-point of the viruses of yellow cucumber mosaic and ordinary cucumber mosaic (10 min. treatment).

Temperature at which extract heated (°C.)	Number of tobacco plants infected of fifteen tested*	
	Yellow cucumber mosaic virus	Ordinary cucumber mosaic virus
65	15	14
68	12	10
69	5	7
70	4	3
71	0	0
72	0	0
75	0	0
Unheated extract	15	15
Uninoculated controls	0	0

* Total of three separate trials.

Table II.

Comparison of tolerance to dilution of the viruses of yellow cucumber mosaic and cucumber mild mosaic with that of ordinary cucumber mosaic virus.

Upper figure, number of tobacco plants tested;
lower figure, number of plants infected.

Dilution of extract	Series I		Series II	
	Yellow cucumber mosaic virus	Ordinary cucumber mosaic virus	Cucumber mild mosaic virus	Ordinary cucumber mosaic virus
Full strength	15* <u>15</u>	15* <u>15</u>	15* <u>15</u>	10† <u>10</u>
1 in 10	15 <u>15</u>	15 <u>15</u>	15 <u>15</u>	10 <u>10</u>
1 in 100	15 <u>15</u>	15 <u>15</u>	15 <u>13</u>	10 <u>10</u>
1 in 1000	15 <u>15</u>	15 <u>15</u>	15 <u>4</u>	10 <u>10</u>
1 in 10,000	15 <u>7</u>	15 <u>7</u>	15 <u>0</u>	10 <u>5</u>
1 in 100,000	15 <u>1</u>	15 <u>2</u>	15 <u>0</u>	10 <u>3</u>
1 in 1,000,000	15 <u>0</u>	15 <u>0</u>	15 <u>0</u>	10 <u>0</u>
Uninoculated controls	15 <u>0</u>	15 <u>0</u>	15 <u>0</u>	10 <u>0</u>

* Total of three separate trials.

† Total of two separate trials.

malformation. On tobacco, in particular, it was observed that the yellow form caused little or no savoying of the leaf, although the ordinary cucumber mosaic virus shows a decided tendency to the production of dark green, blistered areas on this host under certain conditions (Plate II, fig. 1 *B, D*). In the course of the investigation, however, a second yellow strain became differentiated, during a thermal-death-point trial, which consistently caused very marked savoying on tobacco and even more severe chlorosis, the lighter areas of affected leaves becoming almost white (Plate II, fig. 1 *C*). This second strain retained its distinctive characters in subsequent transfers, although in all other respects it was found to be identical with the original yellow form.

Many attempts have been made to purify these yellow strains further from possible traces of ordinary cucumber mosaic virus, but without perceptible effect. Among such attempts may be recorded serial transfers from yellow areas only of infected leaves, according to McKinney's method of purification of yellow tobacco mosaic virus⁽¹²⁾; repeated inoculation of extract at a dilution of 1 in 100,000; and attempts to purify the virus on the basis of possible differential rate of passage through the tobacco plant, according to Koch's method for isolating the potato ring-spot virus⁽¹⁰⁾. Virus recovered at the end of these tests, however, appeared identical in all respects with the original material. On the other hand, artificial mixtures of the yellow and ordinary cucumber mosaic viruses on tobacco could readily be recognised by the resultant symptoms, these representing a gradation of mosaic patterns intermediate between those of ordinary cucumber mosaic and of yellow cucumber mosaic, the amount of yellowing produced varying according to the proportion of the two constituents in the inoculum used. Moreover, inoculation of extract from plants showing intermediate symptoms, at a dilution of 1 in 10,000, served to reveal the two constituents, since under these circumstances some of the inoculated plants developed symptoms of ordinary cucumber mosaic virus and others symptoms characteristic of the original yellow form. Inoculation of the yellow form alone, however, always yielded a constant type of symptom, even at high dilutions, as well as after other treatments applied in property determinations. On the basis of these results it was concluded that the strains of yellow cucumber mosaic virus studied were in essentially "pure" condition.

In an attempt to explain the origin of this form of yellow mosaic, a large number of tobacco plants were inoculated with ordinary cucumber mosaic virus and kept under observation in the greenhouse. In two instances, several weeks after typical symptoms appeared, one plant of a

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large number inoculated developed a single small yellow spot on one of the older leaves, after the manner described by McKinney⁽¹²⁾ and Jensen⁽⁵⁾ in the development of yellow tobacco mosaic¹. By means of needle inoculation from these yellow spots and a series of transfers on tobacco, a form of yellow cucumber mosaic was finally obtained, which, although undoubtedly still containing some unchanged cucumber mosaic virus, bore considerable resemblance to the forms previously described, causing abundant yellowing of the host. It would seem from these results that the ordinary cucumber mosaic virus has a tendency to pass over into a "yellow" form, as has ordinary tobacco mosaic virus, and it is conceivable that some such change occurring in the host plant, followed by recovery of the new form by aphids feeding on the plant, might account for the sudden appearance of the yellow mosaic in the aphid transmission trials recorded above. In whatever manner this yellow cucumber mosaic virus may have arisen, it is evidently to be regarded as a strain variant of ordinary cucumber mosaic virus, and it appears to bear the same relationship to the ordinary form as does yellow tobacco mosaic virus to ordinary tobacco mosaic virus.

The original yellow cucumber mosaic virus, which was exhibited at Ames, Iowa, in December, 1929, in connection with the annual meeting of the American Phytopathological Society, is considered by E. M. Johnson to resemble his cucumber mosaic type 2⁽⁶⁾, though his description of symptoms does not suggest any close similarity. Nevertheless, it seems quite likely that strains of yellow cucumber mosaic virus may occur in different localities where ordinary cucumber mosaic virus is common, although such forms have not been observed by the writer except on the two occasions recorded above.

THE "CUCUMBER MILD MOSAIC VIRUS."

This virus was obtained by Dr J. C. Walker of the Plant Pathology Department, University of Wisconsin, on cucumbers growing in a commercial greenhouse in Milwaukee, Wisconsin, during the fall of 1933. According to Dr Walker, infection appeared to have been carried into the greenhouse by aphids from a summer crop outside, resulting in a severe mosaic on affected plants. Extract from diseased leaves was inoculated to tobacco in order to determine whether the ordinary cucumber mosaic virus (*cucumber virus 1*)⁽⁷⁾ was responsible. The symptoms developing in this host, however, were unlike those of ordinary cucumber mosaic, but

¹ Similar yellow spots have been described by Price (*loc. cit.*), who has isolated several strains of yellow cucumber mosaic virus from them.

consisted of faint chlorotic blotches on an otherwise normal-coloured leaf, or a mild indefinite mottling in which darker areas frequently bordered the veins (Plate II, fig. 2). Such leaves were slightly crinkled, but there was no stunting of the plant and symptoms were absent from the youngest leaves. The incubation period was also one to several days longer than that of ordinary cucumber mosaic. It seemed possible that this virus might represent an attenuated strain of ordinary cucumber mosaic virus, and a further comparison of the two forms was undertaken to determine their relationship.

The virus from Milwaukee, which will be referred to for the present as the "cucumber mild mosaic virus," was found to be readily transmissible by aphids (*M. persicae*) as well as by plant extract (rubbing inoculation). A study of the properties of the two viruses, however, revealed definite differences. Whereas the thermal death-point of ordinary cucumber mosaic virus was found to be above 70° C., that of cucumber mild mosaic virus lay between 60° and 65° C. (Table IV). Similarly, the tolerance to dilution of the latter virus was lower—1 in 1000—(Table II), and the longevity *in vitro*, at about 21° C., between 2 and 4 days—approximately 2 days less than that of ordinary cucumber mosaic virus under the same conditions (Table III). All property tests were made with virus extract from tobacco.

In host range, so far as studied, the two viruses appeared to be similar with one exception, although the symptoms produced by the cucumber mild mosaic virus were always of a much milder type. Thus, on tomato, the only symptom observed was a faint chlorotic blotching of the leaf, with none of the malformation so characteristic of ordinary cucumber mosaic on this host; and on cucumber a conspicuous chlorotic spotting of the leaves occurred without any savoying or leaf distortion and little, if any, stunting or necrosis of the plant (Plate II, fig. 3). Milder symptoms were produced also on *Nicotiana glutinosa* and on spinach. On the other hand, only local infection, in the form of faint chlorotic spots, developed on *Phytolacca decandra*, though the ordinary cucumber mosaic virus readily causes systemic infection of this host, involving severe chlorosis and necrosis of the leaves.

In a brief cytological study of tobacco leaves, infected with cucumber mild mosaic, no inclusion bodies were observed associated with the disease.

From this preliminary investigation, it is concluded that the cucumber mild mosaic virus probably does not represent merely an attenuated form of ordinary cucumber mosaic virus, although the two forms appear to be related in many ways. In view of the differences in their respective

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Table III.

Comparison of longevity in vitro (at about 21° C.) of the viruses of yellow cucumber mosaic and cucumber mild mosaic with that of ordinary cucumber mosaic virus.

Upper figure, number of tobacco plants tested;
lower figure, number of plants infected.

Age of extract in days	Series I		Series II	
	Yellow cucumber mosaic virus	Ordinary cucumber mosaic virus	Cucumber mild mosaic virus	Ordinary cucumber mosaic virus
0	$\frac{15^*}{15}$	$\frac{15^*}{15}$	$\frac{15^*}{15}$	$\frac{10^\dagger}{10}$
1	—	—	$\frac{15}{9}$	$\frac{10}{10}$
2	$\frac{15}{15}$	$\frac{15}{15}$	$\frac{15}{3}$	$\frac{10}{10}$
3	$\frac{15}{15}$	$\frac{15}{15}$	$\frac{15}{2}$	$\frac{10}{8}$
4	$\frac{15}{9}$	$\frac{15}{11}$	$\frac{15}{0}$	$\frac{10}{6}$
5	$\frac{15}{8}$	$\frac{15}{8}$	$\frac{15}{0}$	$\frac{10}{5}$
6	$\frac{15}{0}$	$\frac{15}{3}$	$\frac{15}{0}$	$\frac{10}{0}$
7	$\frac{15}{0}$	$\frac{15}{0}$	$\frac{15}{0}$	$\frac{10}{0}$
Uninoculated controls	$\frac{15}{0}$	$\frac{15}{0}$	$\frac{15}{0}$	$\frac{10}{0}$

* Total of three separate trials.

† Total of two separate trials.

Table IV.

Comparison of thermal death-point of the viruses of cucumber mild mosaic and ordinary cucumber mosaic (10 min. treatment).

Temperature at which extract heated (°C.)	Number of tobacco plants infected of fifteen tested*	
	Cucumber mild mosaic virus	Ordinary cucumber mosaic virus
55	8	15
60	3	15
65	0	15
70	0	4
Unheated extract	14	15
Uninoculated controls	0	0

* Total of three separate trials.

properties, it is felt that they are best regarded, for the present at least, as distinct viruses, though evidently belonging to the same general group. The cucumber mild mosaic virus is definitely distinct from the celery mosaic virus of Doolittle and Wellman⁽²⁾, as regards both symptomatology and properties. While other mosaic diseases of cucumber have been reported, as the "mottled leaf disease" of Jagger⁽⁴⁾, the "cucumber virus 2" mosaic of Porter⁽¹³⁾, and the "Aucuba type of cucumber mosaic" of Bewley⁽¹⁾, it has not been possible to judge from the descriptions given whether any of these forms are similar to the one here described. In symptoms produced on cucumber, however, there appears to be considerable resemblance to the cucumber virus 2 of Porter. It is hoped that the description of the "cucumber mild mosaic virus" given above will make possible a fairly ready identification of this virus.

DISCUSSION.

The purpose of the present paper has been, in part, to describe two viruses of the cucumber mosaic group, which may or may not have been previously recorded, with sufficient completeness to allow subsequent determinations to be made with a reasonable degree of certainty. Failure to determine definitely the relationship of these to certain previously reported forms illustrates again the weakness of attempts to distinguish virus diseases on the basis of symptomatology alone.

The chief interest of this study, however, has lain in the additional evidence furnished of the existence of strains or related forms of viruses and their possible origin. Although no conclusive data are presented here to establish the occurrence of spontaneous variation in this group of pathogenic agents, the recording of data of this sort may be expected to lead eventually to a clearer conception of the nature and behaviour of plant viruses in some such manner as has the study of variation in the bacteria and fungi and other groups of living organisms.

SUMMARY.

A "yellow cucumber mosaic virus" is described, which appeared to develop spontaneously during experiments with ordinary cucumber mosaic on tobacco. This form is readily distinguishable from the ordinary cucumber mosaic virus by the conspicuous bright yellow mottling produced on tobacco and other hosts. In modes of transmission, properties and host range, the virus appears to be identical with ordinary cucumber mosaic virus, and is hence regarded as a strain variant of this form.

A second virus is described on tobacco which appears to belong to the same general group, although showing differences of greater magnitude

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from the ordinary cucumber mosaic virus. This form is distinguished chiefly by the milder type of symptoms produced upon various common hosts, and by its somewhat lower thermal death-point, tolerance to dilution and longevity *in vitro*.

Additional evidence is thus offered of strain variation in the cucumber mosaic group, similar to that already recognised in certain other groups of plant viruses.

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EXPLANATION OF PLATE II.

- Fig. 1. Comparative symptoms of two strains of the yellow cucumber mosaic virus (*B* and *C*) and the ordinary cucumber mosaic virus (*D*) on tobacco. Leaf from control plant (*A*).
- Fig. 2. Comparative symptoms of ordinary cucumber mosaic virus (*B*) and the cucumber mild mosaic virus (*C*) on tobacco. Leaf from control plant (*A*).
- Fig. 3. Comparative symptoms of the cucumber mild mosaic virus (*A*) and the ordinary cucumber mosaic virus (*B*) on cucumber. Leaf from control plant (*C*).

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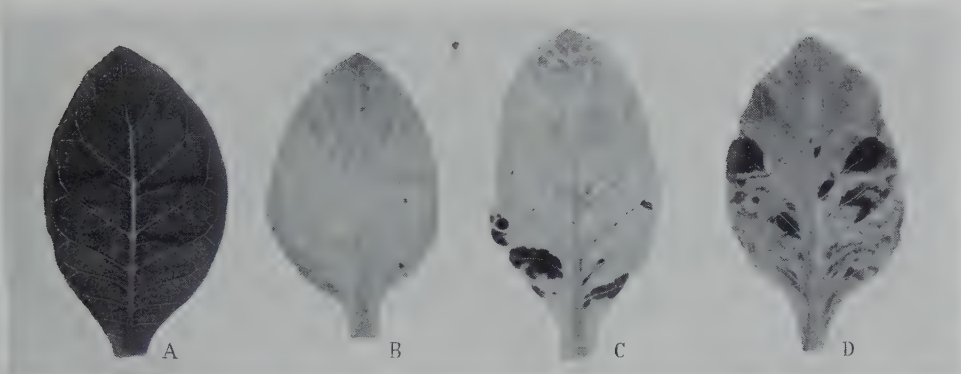


Fig. 1.

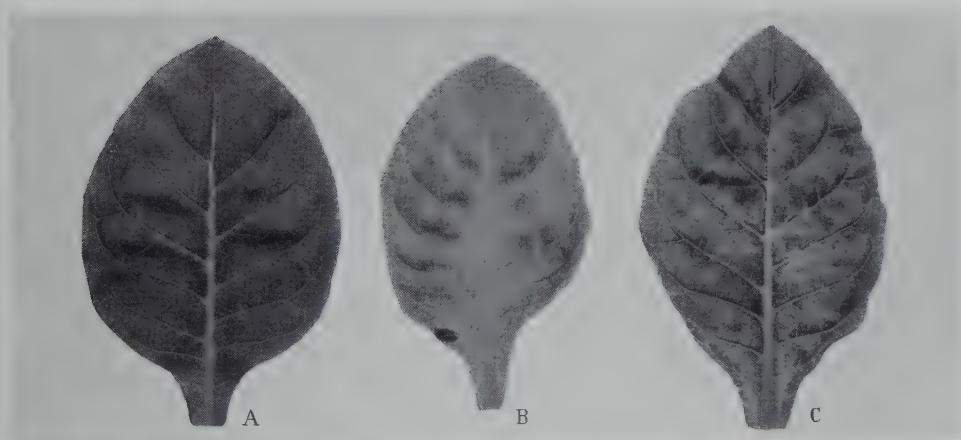


Fig. 2.

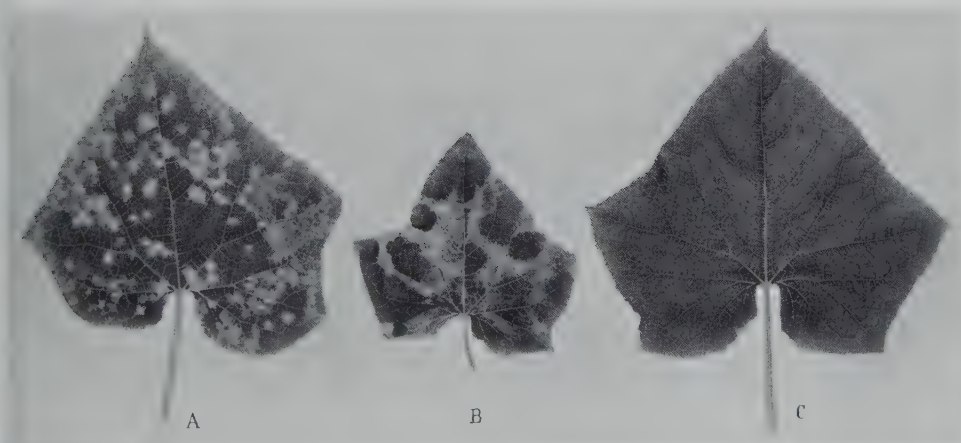


Fig. 3.

A COMPARISON OF CERTAIN FOREIGN AND AMERICAN POTATO VIRUSES¹

BY KARL KOCH AND JAMES JOHNSON.

(*University of Wisconsin.*)

(With Plates III-V.)

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INTRODUCTION.

WITH the reported occurrence of a relatively large number of different potato viruses in various parts of the world, the need for comparative studies in this group of viruses is becoming increasingly evident. The vegetative propagation of the potato, together with the universal culture of this crop, naturally favours a wide distribution of the associated viruses. The striking influence of environment and variety upon symptom expression suggests the possibility that some forms of modification may occur in the viruses themselves. While the present comparative study was undertaken partly for the purpose of securing more information on the possibility of such variation, the chief object has been to study the extent of such synonymy in nomenclature as may exist.

Similar comparative studies have previously been made in a number of instances by other investigators, often with confusing results, owing in

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part to complete reliance upon symptom expression in the potato for purposes of identification. In the present studies, tobacco has been used as much as possible as a test host in addition to potato, and the properties of the viruses themselves have been compared where thought necessary. Although the study is by no means as complete as might be desired, it has demonstrated the striking similarity of certain viruses obtained from widely different sources, and suggests that variation in description and nomenclature is responsible in considerable degree for the present confusion in the potato virus problem.

EARLIER INVESTIGATIONS.

Soon after the recognition of viruses as causal agents of disease in potatoes, Schultz and Folsom (14), Quanjér (9), Murphy (7), and others were able to separate a number of distinct types of virus diseases on this host. Subsequent experience has shown that these disease expressions were for the most part stable, and, except for some synonymy in nomenclature, consequent to almost simultaneous investigations, the subject did not appear to be particularly complicated. With the discovery not only of the frequent occurrence of masked symptoms, due to environmental influences and "carrier" varieties, but also of the regular occurrence of certain viruses in entire stocks of apparently healthy potatoes, at least in the United States, the situation became more involved. It became evident that, in this country at least, in most instances two or more viruses were normally present in virus-diseased potatoes, even though it might be conceded that only one virus was actually concerned with the symptoms expressed. In the case of "rugose mosaic," however, evidence accumulated rapidly from various sources that the typical symptom expression was actually the result of the association of two or more distinct viruses.

The more recent investigations, therefore, have been concerned chiefly with attempts to describe and name the individual viruses which are believed to be concerned with the diseases earlier described. These studies have not cleared the field as rapidly as might be expected, partly on account of the difficulty of, or the failure to, isolate or separate the specific viruses concerned. Consequently we have, for example, a considerable volume of literature dealing with the "mottle" virus (3), subsequently referred to by various writers as the "healthy potato" virus, latent virus, X virus, simple mosaic virus, etc., without either the description, nomenclature or behaviour being differentiated from that of the commonly associated potato ring-spot virus (6).

A detailed review of the literature bearing on these problems is not essential in the present connection, and it will be referred to only as it relates to particular points under consideration. In order adequately to understand the problems concerned, reference should be made to the papers of Quanjer⁽¹⁰⁾, Murphy and McKay⁽⁸⁾, Salaman⁽¹¹⁾, Salaman and Le Pelley⁽¹²⁾, K. M. Smith⁽¹⁵⁾, Burnett and Jones⁽¹⁾, and others.

MATERIALS AND METHODS.

Earlier studies in this laboratory on potato viruses obtained from various sections of the United States were sufficient to furnish the necessary acquaintance with the chief distinguishing characteristics of the various types that occur in this country. It was therefore considered advisable to place most emphasis on viruses that might be obtained from foreign sources. Permission was granted by the Division of Plant Quarantine of the United States Department of Agriculture for the introduction of such potatoes under certain stipulated conditions. Requests for both healthy and virus-diseased tubers were made to pathologists at various foreign institutions. The writers wish to acknowledge their indebtedness to the following, in particular, for the tubers furnished for this investigation: O. Appel, Germany; D. Atanasoff, Bulgaria; V. Lehnovitch, U.S.S.R.; C. J. Magee, Australia; P. A. Murphy, Irish Free State; H. Nakamura, Japan; H. M. Quanjer, Holland; R. N. Salaman, England; G. Samuel, Australia; K. M. Smith, England; and A. Viegas, Brazil.

Some difficulty is naturally encountered, in an investigation of this sort, in obtaining the desired material at the best time for growth and comparison. However, by holding some of the tubers in a low-temperature refrigerator, very little material was lost. Portions of the tubers were planted in fertile soil in 4 in. pots in a thermostatically controlled greenhouse, at a temperature of about 17 to 22° C. The trials, which lasted during two years, were begun in October and continued well into April, when the temperature in the greenhouse becomes too high in Wisconsin for satisfactory work with potatoes. Bliss Triumph potatoes, grown under the same conditions and free from the ordinary virus diseases, but carrying the "mottle" virus and often the potato "ring-spot" virus, were used as stock for inoculation purposes. The Connecticut Havana No. 38 variety of tobacco, grown in a warm greenhouse (27–32° C.), was also used regularly as a test host. Inoculations were made by the rubbing method, and in other details the technique followed was essentially the same as that previously described from this laboratory^(3, 4, 6).

The procedure followed consisted chiefly in inoculating potato and tobacco with extract from plants grown from the tubers obtained for this purpose. If infection of any sort occurred on either host, the virus or viruses thus isolated were compared, as far as possible, with other related forms, with respect to both symptoms and properties. This comparison usually enabled us to make a reliable determination of the specific viruses present in each tuber, except in the case of those not transmissible mechanically by plant extract, with which the present studies are not specially concerned.

EXPERIMENTAL RESULTS.

The results obtained from the inoculation of potato and tobacco with the potato viruses secured from foreign sources are presented in Tables I–IX. While it seems most desirable to give the data in this manner, the results may most profitably be discussed from the standpoint of the individual viruses concerned.

Although inoculations were made to potatoes and to tobacco from plants grown from all tubers, including those plants free from symptoms and subsequently proved to be virus-free, the relatively low number of viruses secured which affected potatoes but not tobacco (such as those of crinkle mosaic, mild mosaic, etc.) was surprising. Consequently, sufficient material did not become available for adequate comparison with certain of the better known American types which are not transmissible to tobacco, and the investigation became limited largely to a study of the “mottle,” “ring-spot,” and “veinbanding” viruses.

(a) *The potato “mottle” virus.*

Particular interest centred round the potato “mottle” virus, which was described by the junior author in 1925(3), and which was shown to be regularly present in all tubers of most, if not all, standard varieties of American potatoes. Subsequent trials in this and other American laboratories (1, 5, 16) have served to substantiate this conclusion. However, it soon became evident that the same situation did not always occur with foreign potato varieties. Not only did the few early trials in our laboratory with certain tubers sent from England fail to yield the mottle virus, but the problem as taken up by European investigators has led to the same conclusion (12). Nevertheless, the occurrence and distribution of the mottle virus in foreign varieties of potatoes has not yet been adequately studied, and complications in nomenclature in this and other countries have not tended to clarify even the data available. The present in-

vestigation could not be carried into sufficient detail to enable us to determine or corroborate the relative degree of infection or freedom from viruses of any particular foreign variety. It is significant, however, as may be seen from an examination of the corresponding tables, that the mottle virus was found in one or more varieties of potato from each of the nine countries from which material was obtained. A compilation of the data shows further that, out of a total of about 75 varieties tested, approximately one-half yielded the mottle virus, the proportion being lowest from Brazil (1 in 5) and highest from Australia (6 in 8).

No evidence developed, on the basis of symptom expression on tobacco, that would justify any belief that the "mottle" virus secured from the various sources was different from the American form. Variations in intensity of symptoms occurred, which may have been due to such differences in virulence as have previously been noted as characteristic of this virus(3). It should be recalled, however, that this virus is commonly associated with the potato ring-spot virus, and, since the separation of the two viruses involves rather lengthy technique, detailed comparison of properties of a number of the "strains" of mottle virus secured from different sources would involve considerable routine.

(b) *The potato "ring-spot" virus.*

The "ring-spot" virus has also been shown in this laboratory to be commonly present in apparently healthy American potatoes, evidently, however, always in association with the mottle virus(3). Recently, the ring-spot virus has been separated from the mottle virus, and the two forms shown to be distinct in properties as well as in symptoms(6). This situation has not been so generally recognised elsewhere, and it is partly for this reason that many of the later names applied to the "mottle" and "ring-spot" viruses are confusing. Furthermore, these viruses are evidently capable of replacing each other, when combined with the vein-banding virus, to produce the symptom expression known as "rugose mosaic" of potato or "spot-necrosis" of tobacco, a situation which, though not without parallel, complicates the problems to be considered.

With these facts in mind, we were not surprised, therefore, to find evidence of the presence of the ring-spot virus in association with practically all cases of mottle in foreign potatoes. The most striking exception appeared to be in the Bulgarian potatoes, where our records indicate the entire absence of ring-spot. Apart from this instance, however, it is evident that ring-spot is quite as widely distributed as is the mottle virus.

A few cases of questionable ring-spot were noted in potatoes from Holland, Ireland, and Russia (Tables VI, VII, IX), suggesting that the range of symptom expression may be wider than that with which we are acquainted in America, or else that still another virus may have been concerned.

It should be stated that the ring-spot virus was not separated from the mottle virus in these studies and the comparisons made are based entirely upon symptom expression. In view of the fact that little is yet known about the influence of the variety of potato and other factors on possible changes in virulence of the ring-spot virus, it is not likely that detailed comparisons would have yielded results of special interest or value in the present connection. On the basis of symptom expression, it is believed safe to say that the potato ring-spot virus, as we have found and described it in American potato varieties, exists in foreign potato varieties in essentially the same manner and relation, though evidently to a lesser extent.

(c) *The potato "veinbanding" virus.*

Despite the fact that the veinbanding virus produces definite symptoms upon both tobacco and certain varieties of potatoes in the absence of other viruses, it evidently escaped detection as an individual entity longer than certain other more rare and obscure forms. The explanation of this lies largely in the potato "rugose mosaic" complex. For all practical purposes, potato "rugose mosaic" is due to the veinbanding virus, and the earlier American investigations on rugose mosaic should be judged from this standpoint. With the discovery of the common occurrence of the mottle and ring-spot viruses in apparently healthy potatoes, and the recognition of another component, necessary for the development of rugose mosaic, which is aphid-transmitted while the former viruses are not, a peculiarly obscure problem was largely solved. However, just as it has already been shown that mottle and ring-spot may evidently replace each other in the virus complex known as rugose mosaic, so it was not entirely clear whether the other necessary components—veinbanding or Smith's virus Y—were one and the same or different viruses. A comparison of this insect-transmitted component, referred to for the present as the "veinbanding virus," as obtained from various sources, should therefore prove to be of particular interest.

The veinbanding virus was found present in potatoes from six of the nine foreign sources from which tubers were tested, the material received from Ireland, Japan, and Russia failing to yield this particular virus,

though it undoubtedly occurs in these countries. While, in America, we are acquainted with this virus on potatoes only as it occurs in the rugose mosaic complex, it was found in apparently "pure" state in potatoes from Holland, Germany, England, Bulgaria, and Australia (Plate III), and from Brazil in combination with leaf-roll only.

Comparative studies of these various "strains" with respect to symptoms on tobacco and potato showed that they were evidently identical in all cases with the exception of the virus Y from England. The Y virus failed, in particular, to yield typical symptoms of American rugose mosaic upon inoculation to Bliss Triumph potato, although it yielded typical spot-necrosis on tobacco. Comparative studies of the properties of the veinbanding virus from Bulgaria, Brazil, Germany, and the United States, and the Y virus from England (Table X), show that the thermal death-point, longevity *in vitro*, and tolerance to dilution of all these forms are essentially the same.

A further comparison of the American veinbanding virus and the English virus Y was undertaken in view of their behaviour on potato, despite the failure of the property tests to show any dissimilarity. A limited study on eight possible differential hosts among the Solanaceae yielded apparently identical behaviour in all instances except possibly one, namely, on *Solanum nigrum* L. This host was susceptible to infection with the veinbanding virus but apparently not susceptible to virus Y. Such a character may eventually prove to be the most simple means of differentiating these two viruses. However, as stated earlier, the differential host reaction is pronounced when the two viruses are inoculated to Bliss Triumph potatoes already containing the mottle virus. The veinbanding virus in this case produces typical rugose mosaic, characterised by rugosity and mottling of the upper leaves, and necrosis and leaf-drop of the lower leaves. Virus Y, under the same conditions, causes necrosis and leaf-drop only, mottling and rugosity of the upper leaves being entirely absent (Plate IV). The symptoms in the latter case are therefore more characteristic of the disease which has been described under the name of "streak" than of rugose mosaic.

Inoculations from various portions of plants affected with these two viruses indicate further that, whereas the veinbanding virus is present in both necrotic and mottled leaves of Bliss Triumph potatoes, virus Y is apparently present only in the necrotic leaves and not in the upper symptomless leaves of the diseased plant.

It may also be noted that slight differential symptoms appear on tobacco when the two viruses are combined with either the mottle or the

ring-spot virus. In this instance, the *Y* virus produces a more serious form of necrosis than does the veinbanding virus, especially in the ring-spot combination. It appears certain, therefore, that Smith's virus *Y*, while possessing many characters in common with the American veinbanding virus, is not identical with it. The American veinbanding virus appears, however, to be more common and universally distributed than is the *Y* form.

(d) *Other potato viruses.*

Apart from the mottle, ring-spot, and veinbanding viruses, a rather surprising scarcity of other types was found in the material secured for these investigations. No viruses, for instance, were detected that could be compared with those of leaf-rolling mosaic, calico, yellow dwarf, spindle tuber, witches' broom, or giant hill. While the above forms were not specially sought for or expected, there is, on the other hand, some reason to believe that the distribution and occurrence of the American mild mosaic and American crinkle mosaic in foreign countries should be greater than has been reported.

Typical crinkle mosaic virus was secured from Australia (Table I) and Japan (Table VIII). This virus was also found in potatoes from Ireland (Table VII) reported as affected with "crinkle," and in potatoes from England reported as containing "crinkle *A*" (Table IV). Murphy's virus *A*, transferred alone to American Bliss Triumph, also yielded crinkle mosaic in our trials (Table VII). Since virus *A* is said to be a part of the "crinkle" complex⁽²⁾, these results are to be expected on the assumption that the symptoms of American crinkle mosaic are a consequence of the presence of two viruses, *i.e.* virus *A* and the "mottle" virus. Similarly, the relationship of Salaman's para-crinkle and the American mild mosaic or crinkle mosaic may be suspected. It is important, however, to note here that neither the American mild mosaic nor crinkle mosaic viruses or complexes are transmissible as such to tobacco, nor to other solanaceous plants, as far as is known. On the other hand, the recent discussions of the "crinkle" complex from Europe⁽²⁾ suggest that the European "crinkle" is, in part at least, similar to, if not identical with, American "rugose mosaic." It is not at all unlikely, therefore, that the presence in Europe of viruses such as those of crinkle mosaic and mild mosaic, which are easily masked by environmental conditions, very sensitive to the variety of potato, and not transmissible to tobacco, may have served to confuse the problem. This supposition is further supported by the uncertainty regarding the use of the terms "mosaic," "simple mosaic," "common

mosaic," etc. These diseases are said to be symptom expressions of the American mottle and ring-spot (English *X*) viruses, whereas in America it yet remains to be shown that these viruses produce any symptoms whatever of a qualitative sort on potatoes. It may also be stated that American crinkle mosaic and rugose mosaic are often very similar in symptom expression if circumstances are such that necrosis and leaf-drop are not favoured in the latter disease. Unfortunately, the nomenclature (*i.e.* "crinkle" as distinct from "crinkle mosaic") has also to some extent added to the confusion. Still another situation apparently exists in European potato stocks, which possibly does not occur at all in standard stocks of American potatoes, namely, the presence of the vein-banding virus alone as the cause of a specific disease in certain varieties, or as a "carried" virus in other tolerant varieties. The term "mosaic" as used in Europe may therefore represent a number of distinct diseases, some or all of which may appear to yield symptoms on tobacco unless due cognisance is taken of the presence of other viruses which do not, or may not, yield symptoms on potatoes.

The potato "streak" and "leaf-drop" diseases remain obscure as far as definite connection with any particular viruses is concerned. These terms, like "mosaic," hence have little significance beyond denoting a particular type of symptom expression. Certainly the rugose mosaic complex, or the *X* and *Y* combination, is responsible for many cases previously described as streak, leaf-drop, and stipple streak. That still other viruses may be involved, either alone or in combination, must be admitted. In our present investigation we have come across one such virus in potatoes of the variety President secured from Ireland. This virus may perhaps be identical with such previously reported viruses as those of top-necrosis(10), potato necrosis(13), Up-to-Date streak(8), etc., but this cannot be verified on account of incomplete descriptions. Hence this virus is now tentatively described as "potato streak virus" (Plate V). It was concluded that this virus was not aphid-transmissible following trials with fifty plants, using *Myzus persicae* Sulz. and *Macrosiphum solanifolii* Ashm. as possible vectors. The remainder of the data on which the description of this streak virus is based is presented in Table XI.

Potato streak virus. Not transmitted by *Myzus persicae* Sulz. or *Macrosiphum solanifolii* Ashm. Transmissible mechanically by plant extract. Resistance to ageing *in vitro* between 4 and 6 days at about 22° C. Thermal death point between 55 and 58° C. (10 min.). Primary infection causes distinct necrotic, streak-like symptoms on leaf veins, lamina and stems of potato (Bliss Triumph and Green Mountain varie-

ties), generally resulting in killing of the bud first, followed by a downward necrosis which may kill the entire plant. No mottling on potato. Tobacco susceptible, symptoms consisting of an indefinite and mild necrosis in the form of irregular white blotches. Host range and distribution unknown.

DISCUSSION OF RESULTS.

The entire purpose of the present studies could not be carried to completion for various reasons. Many questions remain to be answered; but, in addition to such details as have been noted, the writers have been impressed first of all with the remarkable degree with which the viruses collected from the various parts of the world have retained their identity, after being separated from a common origin, in some instances at least, for perhaps a century or more. That some minor degree of variation has occurred in rare instances is admitted, and as an example of this may be cited the differences in symptomatology between the veinbanding virus and Smith's *Y* virus, in spite of their striking similarity in other more important respects.

On the other hand, it seems to us that convincing evidence has been offered to show that one of the chief problems confronting the potato virus student at present is that of determining the existing synonymy in virus description and nomenclature. Peculiarly enough, it is with the small group of potato viruses that should most easily be identified that the greatest difficulty appears to exist, namely, with that group which is transmissible to tobacco and other solanaceous plants. These difficulties, we are convinced, are due chiefly to two reasons, namely, the failure to determine the characteristics of the virus itself, and, secondly, to an easy tendency to apply new names without adequate consideration of previous work.

SUMMARY.

Potato viruses obtained from nine foreign countries have been compared with typical American forms. The chief emphasis has been placed on the potato "mottle," "ring-spot," and "veinbanding" viruses.

The mottle and ring-spot viruses were found in potatoes from all nine foreign countries, but only in about one-half of the 75 varieties or lots tested. Hence these viruses appear to be less widespread in foreign than in standard American potato varieties. The veinbanding virus was found in potatoes from six of the nine foreign countries, and was often free from associated viruses.

The remarkable constancy of the viruses secured from widely different sources, when compared under identical conditions, was outstanding. Some degree of variation was found in certain cases, as for example between the veinbanding virus and the Y virus from England, though these viruses are very similar in most respects.

The possible relationships of certain other potato viruses are discussed, and a potato streak virus is briefly described.

Incomplete or unsatisfactory descriptions and synonymy in nomenclature are believed to be largely responsible for the existing confusion in potato virus literature. These difficulties may largely be overcome by devoting more attention to the description of the viruses themselves.

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Table I.

A summary of results from inoculations with seven varieties of potatoes obtained from Australia.

Potato variety, source of inoculum	Reported as	Symptoms expressed	Inoculations on potato		Inoculations on tobacco		Viruses found present
			Number showing infection	Symptoms expressed	Number infected	Symptoms expressed	
Big Top Brownell	Leaf-roll	Leaf-roll	$\frac{5^*}{0}$	None	$\frac{20}{0}$	None	Leaf-roll†
Carmen 1	Healthy	Crinkling, mottling	$\frac{10}{10}$	Rugose mosaic	$\frac{20}{20}$	Vein-banding	Veinbanding
Carmen 2	Healthy	None	$\frac{10}{0}$	None	$\frac{20}{20}$	Mottle, ring-spot	Mottle, ring-spot
Coronation	Mosaic 1	Crinkling, mottling	$\frac{10}{3}$	Crinkle mosaic	$\frac{20}{20}$	Mottle, ring-spot	Mottle, ring-spot, crinkle mosaic
Coronation	Mosaic 2	Crinkling, mottling	$\frac{10}{4}$	Crinkle mosaic	$\frac{20}{20}$	Mottle, ring-spot	Mottle, ring-spot, crinkle mosaic
Delaware	Healthy	None	$\frac{10}{0}$	None	$\frac{20}{20}$	Mottle, ring-spot	Mottle, ring-spot
Snow Flake	Healthy	None	$\frac{10}{0}$	None	$\frac{20}{20}$	Mottle, ring-spot	Mottle, ring-spot
Symington	Leaf-roll	Leaf-roll	$\frac{5}{0}$	None	$\frac{20}{0}$	None	Leaf-roll†
Up-to-Date	Healthy	None	$\frac{10}{0}$	None	$\frac{20}{20}$	Mottle	Mottle
Up-to-Date	Leaf-roll	Leaf-roll	$\frac{5}{0}$	None	$\frac{20}{0}$	None	Leaf-roll†

* Upper figure, number of plants inoculated; lower figure, number infected.

† Conclusion based on symptoms on original plants.

Table II.

A summary of results from inoculations with five varieties of potatoes obtained from Brazil.

Potato variety, source of inoculum	Reported as	Symptoms expressed	Inoculations on potato		Inoculations on tobacco		Viruses found present
			Number showing infection	Symptoms expressed	Number infected	Symptoms expressed	
Ouro 1 (Gold)	Healthy	None	$\frac{5}{0}$	None	$\frac{20}{0}$	None	None
Ouro 2 (Gold)	Healthy	None	$\frac{5}{0}$	None	$\frac{20}{0}$	None	None
Rio Grande Manchada	Leaf-roll	Crinkling, mottling, leaf-roll	$\frac{10}{10}$	Rugose mosaic	$\frac{20}{20}$	Vein-banding	Veinbanding, leaf-roll*
King Edward	Leaf-roll	Crinkling, mottling, leaf-roll	$\frac{10}{8}$	Rugose mosaic	$\frac{20}{20}$	Spot-necrosis (severe)	Veinbanding, mottle, ring-spot, leaf-roll*
Up-to-Date	Leaf-roll	Crinkling, mottling, leaf-roll	$\frac{10}{9}$	Rugose mosaic	$\frac{20}{20}$	Vein-banding	Veinbanding, leaf-roll*
Up-to-Date	Mosaic	Leaf-roll	$\frac{10}{0}$	None	$\frac{20}{0}$	None	Leaf-roll*
Wild Variety	Leaf-roll	Crinkling, mottling, leaf-roll	$\frac{10}{10}$	Rugose mosaic	$\frac{20}{20}$	Vein-banding	Veinbanding, leaf-roll*

* Conclusion based on symptoms on original plants.

Table III.

A summary of results from inoculations with sixteen varieties of potatoes obtained from Bulgaria.

Potato variety, source of inoculum	Symptoms expressed	Inoculations on potato		Inoculations on tobacco		Viruses found present
		Number showing infection	Symptoms expressed	Number infected	Symptoms expressed	
Austrian variety	None	5 0	None	20 0	None	None
Bintje	None	5 0	None	20 0	None	None
Bulgarian 31	Mottling, rugosity, leaf-drop	10 10	Rugose mosaic	20 20	Spot-necrosis	Veinbanding, mottle
Bulgarian 63	None	10 0	None	20 0	None	None
Bulgarian 100	None	10 0	None	20 0	None	None
Early Rose	None	5 0	None	20 20	Mottle	Mottle
Great Scot	None	5 0	None	20 20	Mottle	Mottle
Institut de Beauvais	None	10 0	None	20 0	None	None
Kerr's Pink	None	10 10	Leaf-drop	20 20	Y	Y
King Edward	None	5 0	None	20 0	None	None
Koksiaan	Mottling, rugosity	5 5	Rugose mosaic	20 20	Veinbanding	Veinbanding
Litavo	None	10 0	None	20 0	None	None
Magnum Bonum	None	5 0	None	20 0	None	None
May Queen	None	5 0	None	20 20	Mottle	Mottle
Paul Kruger	None	5 0	None	20 0	None	None
Unknown (X)	Mottling, leaf-drop	10 9	Rugose mosaic	20 20	Spot-necrosis	Veinbanding, mottle

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Table IV.

A summary of results from inoculations with four varieties of potatoes obtained from England. (First eight lots from Dr R. N. Salaman, last two lots from Dr K. M. Smith.)

Potato variety, source of inoculum	Reported as	Symptoms expressed	Inoculations on potato		Inoculations on tobacco		Viruses found present
			Number showing infection	Symptoms expressed	Number infected	Symptoms expressed	
Arran Victory	Healthy	None	$\frac{10}{0}$	None	$\frac{20}{0}$	None	None
Arran Victory	Para-crinkle	Mottling, crinkling	$\frac{10}{10}$	Mottling, crinkling	$\frac{20}{0}$	None	Mild mosaic?
Di Vernon	Top-necrosis	Mottling	$\frac{10}{10}$	Slight mottling	$\frac{20}{0}$	None	Mild mosaic?
President	Healthy	None	$\frac{10}{0}$	None	$\frac{20}{0}$	None	None
President	Crinkle A	Mottling, crinkling	$\frac{10}{10}$	Mottling, crinkling	$\frac{20}{20}$	Mottle, ring-spot	Mottle, ring-spot, crinkle mosaic?
President	Y	Mottling, rugosity, leaf-drop	$\frac{10}{10}$	Rugose mosaic	$\frac{20}{20}$	Vein-banding	Veinbanding
President	X	None	$\frac{10}{0}$	None	$\frac{20}{20}$	Mottle, ring-spot	Mottle, ring-spot
Up-to-Date	Top-necrosis	Mottling?	$\frac{10}{10}$	Slight mottling	$\frac{20}{20}$	Mottle	Mottle, mild mosaic?
President	X	None	$\frac{15}{0}$	None	$\frac{25}{25}$	Mottle, ring-spot	Mottle, ring-spot
Arran Victory	Y	Mottling rugosity	$\frac{15}{8}$	Leaf-drop	$\frac{25}{25}$	Vein-banding	Y

Table V.

A summary of results from inoculations with four varieties of potatoes obtained from Germany.

Potato variety, source of inoculum	Symptoms expressed	Inoculations on potato		Inoculations on tobacco		Viruses found present
		Number showing infection	Symptoms expressed	Number infected	Symptoms expressed	
Ackersegen	Mottling, rugosity, leaf-drop	$\frac{10}{10}$	Rugose mosaic	$\frac{10}{10}$	Spot-necrosis (severe)	Veinbanding, mottle, ring-spot
Ackersegen	None	$\frac{10}{0}$	None	$\frac{25}{0}$	None	None
Blaue Gelb-fleischige	Leaf-drop	$\frac{10}{10}$	Rugose mosaic	$\frac{10}{10}$	Spot-necrosis (severe)	Veinbanding, mottle, ring-spot
Blaue Gelb-fleischige	None	$\frac{10}{0}$	None	$\frac{25}{25}$	Mottle and ring-spot	Mottle, ring-spot
Dauerragis	Mottling, rugosity	$\frac{10}{10}$	Rugose mosaic	$\frac{10}{10}$	Veinbanding	Veinbanding
Dauerragis	None	$\frac{10}{0}$	None	$\frac{25}{0}$	None	None
Erdgold	Mottling, rugosity	$\frac{10}{9}$	Rugose mosaic	$\frac{10}{10}$	Veinbanding	Veinbanding
Erdgold	None	$\frac{10}{0}$	None	$\frac{10}{0}$	None	None

Table VI.

A summary of results from inoculations with six varieties of potatoes obtained from Holland.

Potato variety, source of inoculum	Reported as	Symptoms expressed	Inoculations on potato		Inoculations on tobacco		Viruses found present
			Number showing infection	Symptoms expressed	Number infected	Symptoms expressed	
Alpha	Acronecrosis (Smith's X)	Mottling	$\frac{10}{5}$	Slight mottling	$\frac{20}{20}$	Mottle, ring-spot	Mottle, ring-spot, mild mosaic?
Bravo	Simple mosaic	Mild mottling	$\frac{10}{7}$	Faint mottling	$\frac{20}{0}$	None	Mild mosaic?
Bravo	Interveinal mosaic	None	$\frac{10}{7}$	Leaf-drop	$\frac{20}{20}$	Mottle, ring-spot	Mottle, ring-spot, and unidentified virus
Bravo	Mosaic?	Mottling	$\frac{10}{4}$	Mottling	$\frac{20}{20}$	None	Mild mosaic?
Institut de Beauvais	Acropetal necrosis (Smith's Y) (rugose mosaic)	Mottling, rugosity, leaf-drop	$\frac{10}{10}$	Rugose mosaic	$\frac{20}{20}$	Vein-banding	Veinbanding
Magdeburger Blaue	Acronecrosis (Smith's X)	None	$\frac{10}{8}$	Leaf-drop	$\frac{20}{20}$	Mottle, ring-spot?	Mottle, ring-spot?, and unidentified virus
Monocraat	Virus A? Smith's X?	None	$\frac{10}{9}$	Leaf-drop, mottling	$\frac{20}{20}$	Ring-spot?	Ring-spot?, and unidentified virus
Zeeland	Acropetal necrosis (Smith's Y)	Mottling	$\frac{10}{5}$	Leaf-drop	$\frac{20}{20}$	Y	Y

Table VII.

A summary of results from inoculations with four varieties of potatoes obtained from Irish Free State.

Potato variety, source of inoculum	Reported as	Symptoms expressed	Inoculations on potato		Inoculations on tobacco		Viruses found present
			Number showing infection	Symptoms expressed	Number infected	Symptoms expressed	
Arran Victory	Healthy	None	$\frac{10}{0}$	None	$\frac{25}{0}$	None	None
Irish Chieftain	Virus A	None	$\frac{15}{12}$	Mottling, crinkling	$\frac{25}{0}$	None	Crinkle mosaic
President	Healthy	None	$\frac{10}{0}$	None	$\frac{25}{25}$	Mottle, ring-spot	Mottle, ring-spot
President	Crinkle	Mottling, crinkling	$\frac{15}{4}$	Mottling, crinkling	$\frac{25}{25}$	Mottle, ring-spot	Mottle, ring-spot, crinkle mosaic
President	Interveinal mosaic	Necrotic areas between veins	$\frac{15}{13}$	Leaf-drop	$\frac{25}{25}$	Mottle, ring-spot	Mottle, ring-spot?, and "streak"
President	Simple mosaic	None	$\frac{15}{0}$	None	$\frac{25}{25}$	Mottle	Mottle
Up-to-Date	Streak-free	None	$\frac{10}{0}$	None	$\frac{25}{25}$	Mottle	Mottle
Up-to-Date	Streak	None	$\frac{10}{0}$	None	$\frac{25}{25}$	Mottle, ring-spot	Mottle, ring-spot

Table VIII.

A summary of results from inoculations with two varieties of potatoes obtained from Japan.

Potato variety, source of inoculum	Reported as	Symptoms expressed	Inoculations on potato		Inoculations on tobacco		Viruses found present
			Number showing infection	Symptoms expressed	Number infected	Symptoms expressed	
New Japanese variety	Healthy	None	$\frac{10}{0}$	None	$\frac{25}{0}$	None	None
Snow Flake	Diseased	Mottling, crinkling	$\frac{10}{4}$	Mottling, crinkling	$\frac{25}{25}$	Mottle, ring-spot	Mottle, ring-spot, crinkle mosaic

Table IX.

A summary of results from inoculations with twenty-six varieties of potatoes obtained from U.S.S.R.

Potato variety, source of inoculum	Symptoms expressed	Inoculations on potato		Inoculations on tobacco		Viruses found present
		Number showing infection	Symptoms expressed	Number infected	Symptoms expressed	
Asia B	None	$\frac{5}{0}$	None	$\frac{20}{20}$	Mottle, ring-spot	Mottle, ring-spot
Beauty of Hebron	None	$\frac{5}{0}$	None	$\frac{20}{20}$	Mottle	Mottle
Belladonna	None	$\frac{10}{0}$	None	$\frac{20}{20}$	Mottle, ring-spot	Mottle, ring-spot
Centeneri	None	$\frac{5}{0}$	None	$\frac{20}{20}$	Mottle, ring-spot?	Mottle, ring-spot?
Centifolia	None	$\frac{10}{0}$	None	$\frac{20}{20}$	Mottle, ring-spot	Mottle, ring-spot
Cuckuck	None	$\frac{10}{0}$	None	$\frac{20}{20}$	Mottle, ring-spot	Mottle, ring-spot
Deodara	None	$\frac{5}{0}$	None	$\frac{20}{0}$	None	None
Early Rose	None	$\frac{5}{0}$	None	$\frac{20}{20}$	Mottle	Mottle
Epicure	None	$\frac{5}{0}$	None	$\frac{20}{20}$	Ring-spot?	Ring-spot?
Great Scot	None	$\frac{10}{0}$	None	$\frac{20}{0}$	None	None
Jubel	None	$\frac{10}{0}$	None	$\frac{20}{20}$	Mottle, ring-spot	Mottle, ring-spot
Korenevski	None	$\frac{5}{0}$	None	$\frac{20}{0}$	None	None
Kruger	None	$\frac{5}{0}$	None	$\frac{20}{0}$	None	None
Lorch	None	$\frac{5}{0}$	None	$\frac{20}{0}$	None	None
Maercker	None	$\frac{5}{0}$	None	$\frac{20}{0}$	None	None

Table IX (*cont.*)

Potato variety, source of inoculum	Symptoms expressed	Inoculations on potato		Inoculations on tobacco		Viruses found present
		Number showing infection	Symptoms expressed	Number infected	Symptoms expressed	
Ohio 2	None	$\frac{10}{0}$	None	$\frac{20}{0}$	None	None
Parnassia	None	$\frac{5}{0}$	None	$\frac{20}{0}$	None	None
Rudzinski	None	$\frac{5}{0}$	None	$\frac{20}{0}$	None	None
Sass	None	$\frac{5}{0}$	None	$\frac{20}{20}$	Mottle, ring-spot	Mottle, ring-spot
Schestinedelnyi	None	$\frac{5}{0}$	None	$\frac{20}{20}$	Mottle, ring-spot	Mottle, ring-spot
Silesia	None	$\frac{5}{0}$	None	$\frac{20}{0}$	None	None
Snejinka 2	None	$\frac{5}{0}$	None	$\frac{20}{20}$	Mottle	Mottle
Snejinka 3	None	$\frac{5}{0}$	None	$\frac{20}{20}$	Mottle	Mottle
Sol. 414	None	$\frac{5}{0}$	None	$\frac{20}{0}$	None	None
Vermont	None	$\frac{10}{0}$	None	$\frac{20}{20}$	Mottle	Mottle
Wohltman	None	$\frac{5}{0}$	None	$\frac{20}{0}$	None	None

Table X.

*Comparison of properties of viruses of the veinbanding type
obtained from various countries.*

		Source of virus and number of tobacco plants infected out of ten inoculated				
Property	Treatment	Bulgaria	Brazil	Germany	United States	England (Smith's Y)
Thermal death-point °C.	Unheated control	10	10	10	10	10
	50	10	9	10	10	10
	55	4	5	7	3	5
	58	1	2	2	1	1
	60	0	0	0	0	0
Tolerance to dilution	None	10	10	10	10	10
	1-100	10	10	10	10	10
	1-1,000	4	5	4	5	4
	1-5,000	2	2	1	2	2
	1-10,000	0	0	0	0	0
Longevity <i>in vitro</i> (days)	None	10	10	10	10	10
	2	7	5	5	7	4
	4	1	1	0	1	0
	6	0	0	0	0	0
	8	0	0	0	0	0

Table XI.

Determination of properties of the potato streak virus.

Temperature °C.	Thermal death-point		Ageing in days	Longevity <i>in vitro</i>		Dilution	Tolerance to dilution	
	Number of plants infected out of fifteen inoculated			Number of plants infected out of fifteen inoculated			Number of plants infected out of fifteen inoculated	
	Tobacco	Potato		Tobacco	Potato		Tobacco	Potato
Unheated control	14	6	None	14	6	None	—	8
50°	14	8	2	11	3	1-10	11	7
53°	11	4	4	2	0	1-100	10	8
55°	5	2	6	0	0	1-1,000	5	0
58°	0	0	8	0	0	1-10,000	0	0
60°	0	0	10	0	0	1-100,000	0	0

EXPLANATION OF PLATES III—V.

Plate III. Typical leaves of the variety Carmen as secured from Australia. (A) From plant carrying the mottle and ring-spot viruses only. (B) From plant containing the veinbanding virus only.

Plate IV. Comparative inoculations with Smith's Y virus (B), and the veinbanding virus (C) to the American Bliss Triumph variety (carrying mottle). Healthy control (A).

Plate V. The potato streak virus, originally secured from the variety President from Ireland, showing different stages of necrosis on the variety Bliss Triumph.

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KOCH AND JOHNSON.—A COMPARISON OF CERTAIN FOREIGN AND AMERICAN
POTATO VIRUSES (pp. 37-54).



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MOSAIC DISEASES OF THE CUCUMBER*

BY G. C. AINSWORTH, B.Sc., PH.D.

(*Experimental and Research Station, Cheshunt, Herts.*)

(With Plates VI–VIII and 2 Text-figures.)

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INTRODUCTION.

THE first authentic record of cucumber mosaic in this country was that of Bewley and Buddin(3) in 1921. Bewley(2) recognised two types of cucumber mosaic which have been distinguished as mild or ordinary mosaic and aucuba mosaic. The mild form caused distortion of the foliage, a leaf-mottle restricted to varying shades of light and dark green with occasional yellow areas and stunting of the plant. The fruit was rarely affected. In the aucuba form distortion of the leaves was not pronounced but there was a distinct type of light yellow mottle and the fruit was liable to be marked. These diseases were not transmissible to solanaceous plants. A third mosaic disease, with a wider host range, characterised by a yellow mottle of cucumber foliage and fruit has recently appeared. This last mosaic is caused by *cucumber virus* 1 (Johnson(12)), the virus responsible for the common cucumber mosaic of America.

The above three diseases and the causal viruses are described and discussed in this paper.

* This paper includes part of a thesis approved by the University of London for the Degree of Doctor of Philosophy.

NOMENCLATURE.

The mild mosaic mentioned above is the cucumber mosaic most frequently recorded at this station and is the disease usually known here as "cucumber mosaic," but in the literature the term cucumber mosaic, unqualified, is generally applied to the disease caused by *cucumber virus* 1.

It is thought desirable that a disease name should, if possible, give an indication of the typical disease symptoms and bearing this in mind the following names are proposed for the three diseases:

- (1) *Green-mottle mosaic* of cucumber for the mild or ordinary mosaic.
- (2) *Yellow mosaic* of cucumber for the "aucuba" mosaic.
- (3) *Yellow-mottle mosaic* of cucumber for the disease caused by *cucumber virus* 1.

The use of the synonyms given should be discontinued.

There is no reason why calling the common cucumber mosaic of America yellow-mottle mosaic in this country should lead to any confusion provided the name of the causal virus is not duplicated and if in technical writing the name of the *virus* is stated.

METHODS.

All the experimental work was carried out in a glasshouse kept free from insects by fumigation and care was taken to prevent accidental infections during cultural operations. Plants were raised from seed in one compartment of the house and after inoculation kept under observation for a month or longer in smaller chambers of the same glasshouse. Plants were usually grown in 3-in. pots but when necessary 6-in., or larger, pots were used. All experiments were repeated at least once.

DESCRIPTION OF THE DISEASES AND THE VIRUSES.

(1) Green-mottle mosaic of cucumber.

Disease synonyms. Green, mild, or ordinary mosaic, "cucumber mosaic."

Virus. *Cucumber virus* 3.

Host range. Cucurbitaceae. (The virus has only been found occurring naturally on cucumber.)

Disease symptoms.

Cucumis sativus, cucumber (variety, Butcher's Disease Resister), is readily infected by mechanical inoculation. Rubbing the leaves with a pad of muslin moistened with infected juice results in 100 per cent. infection unless the juice be largely diluted. Symptoms appear seven to

fourteen days after inoculation as a slight clearing of the veins and crumpling of the young leaves, followed by a light green-dark green mottle, together with blistering and distortion of the leaves (see Plate VI, fig. 1) and stunting of the plant. The symptoms first appear on the younger leaves, and leaves fully developed at the time of inoculation show no symptoms. The mottle is independent of the season but leaf-distortion is more severe when a plant is growing slowly in winter (see Plate VI, fig. 2). Occasional yellow flecks occur on leaves showing the green mottle and may on fully developed leaves be a prominent symptom. The fruit is usually unmarked though it may be slightly mottled.

Cucumis melo (melon), *C. anguria* (gherkin) and *C. maderaspatanus* (seed from Poona) have been infected. Symptoms on all: a dark green-light green mottle of varying intensity, leaf-distortion and stunting of the plant.

Citrullus vulgaris, water-melon (varieties Florida Favourite and Dixie), seedlings are easily infected. Symptoms: a dark green-light green mottle (see Plate VI, fig. 3) and slight stunting.

Cucurbita pepo (vegetable marrow) and *Bryonia dioica* (common bryony) have not been infected and all attempts to infect solanaceous plants have failed.

Properties of cucumber virus 3.

Filterability. Can be filtered unchanged through Pasteur-Chamberland filters, L 1-L 7.

Resistance to ageing in vitro. One year or longer.

Resistance to heat. Survives 80° C. for 10 min. but inactivated by 90° C. for 10 min.

Resistance to alcohol (C₂H₅.OH). Not inactivated by 50 per cent. alcohol in 1 hour.

(2) Yellow mosaic of cucumber.

Disease synonyms. "Aucuba" mosaic of cucumber, "cucumber mosaic."

Virus. *Cucumber virus 4.*

Host range. Cucurbitaceae. (The virus has only been found occurring naturally on cucumber.)

Disease symptoms.

Cucumis sativus, cucumber (variety Butcher's Disease Resister), is readily infected by mechanical inoculation. The incubation period is nine to sixteen days, *i.e.* slightly longer than for green-mottle mosaic. The first symptoms, a slight clearing of the veins and temporary crumpling of

the apical leaves, are followed by a bright yellow mottle, but little or no distortion of the leaves, and the plant is slightly stunted. The mottle either takes the form of few to very many star-like spots which may almost cover the entire leaf (see Plate VII, fig. 4) or gives a vein-banding effect. The vein-banding may be well defined (Plate VII, fig. 5) or appear as a filigree of fine lines which follow the smaller veins. The colour of the mottle, which is always well defined, varies from a pale yellow or greenish cream to nearly white on older leaves. Under less favourable growing conditions the bright yellow mottle is replaced by a rather inconspicuous yellowish-green mottle (see Plate VII, fig. 6) and slight distortion when the symptom picture is very similar to that given by a plant infected with *cucumber virus* 3. This led to the two viruses being confused in a preliminary account given by the writer⁽¹⁾ of part of the work here reported. The fruit is marked by yellow or silver coloured spots or streaks (see Plate VII, fig. 7) and, according to unpublished observations of W. F. Bewley, fruit marking is most severe at temperatures higher than 84° F.

C. melo, melon, has been infected and shows similar symptoms to cucumber.

Citrullus vulgaris, water-melon (varieties Florida Favourite and Dixie), seedlings can be infected and exhibit a yellow mottle of the leaves (see Plate VII, fig. 8) and slight stunting of the plant.

All attempts to infect solanaceous plants (tobacco, tomato, *N. glutinosa*, and *Datura Stramonium* were tried) have failed.

Properties of cucumber virus 4.

Filterability. Can be filtered unchanged through Pasteur-Chamberland filters, L 1–L 7.

Resistance to ageing in vitro. Nine months or longer.

Resistance to heat. Survives 80° C. for 10 min. but inactivated by 90° C. for 10 min.

Resistance to alcohol (C₂H₅.OH). Not inactivated by 50 per cent. alcohol in 1 hour.

(3) Yellow-mottle mosaic of cucumber.

This disease has only recently been noticed in England. It was found on cucumber for the first time by the writer in 1932. Besides cucumber the virus has been isolated from naturally infected vegetable marrow, tomato, gherkin, *Bryonia alba* (white bryony)¹ (see Plate VIII, fig. 10), *Hyoscyamus* sp. and *Datura Stramonium*. (The two last named plants

¹ Not indigenous to England. Found in a garden.

were growing as weeds adjacent to a plot of infected gherkins.) Although at present yellow-mottle mosaic does not appear to occur very frequently in commercial cucumber nurseries, it has proved a serious disease in America and is potentially a serious one in this country, particularly as its host range includes the tomato and other solanaceous plants.

The virus was examined and considered to be similar, if not identical, to *cucumber virus* 1 of Johnson⁽¹²⁾. Dr Henderson Smith very kindly supplied some authentic American cucumber mosaic virus material with which the English virus has been compared and found to agree closely. Unless stated to the contrary the descriptions given below of the virus and the symptoms produced on different hosts apply to both English and American strains.

Disease synonyms. Yellow mosaic (Ainsworth⁽¹⁾) and the American names common cucumber mosaic, cucurbit mosaic and white pickle.

Virus. *Cucumber virus* 1 (Johnson⁽¹²⁾).

Host range. Cucurbitaceae, Solanaceae and others.

Literature. There is a considerable literature dealing with this virus. Doolittle⁽⁵⁾ described the type disease on which J. Johnson⁽¹²⁾ based his description of *cucumber virus* 1. E. M. Johnson⁽¹¹⁾ differentiated three types by the reactions of tobacco and Price⁽²⁰⁾ has shown that numerous strains of this virus exist and obtained evidence that they arise by mutation or a similar process.

Disease symptoms.

Cucumis sativus, cucumber (variety Butcher's Disease Resister), can be infected at all stages of its growth. Systemic symptoms appear six to ten days after inoculation when young plants are used, but the period is slightly longer with older plants. The first symptom may be the development of more or less circular yellowish areas on the leaves inoculated or pale green spots on the cotyledons, if inoculated, three or four days after inoculation. The characteristic symptoms are a yellow mottle on all leaves developed after inoculation (see Plate VIII, fig. 9), some leaf-distortion and stunting of the plant. The fruit is usually mottled (see Plate VIII, fig. 11). More severe fruit symptoms are occasionally met with under commercial conditions when the fruits are misshapen and nearly white in colour with irregular green areas. This symptom is similar to the "white pickle" symptom of fruit described and figured by Doolittle⁽⁵⁾ but it has not been obtained under the experimental conditions. The yellow leaf mottle is rather different in colour and type from that produced by *cucumber virus* 4. It is of a greenish yellow colour and on well-developed

leaves rather diffuse, the green parts of the leaf yellowing slightly, while the yellow mottle due to *cucumber virus 4* is more sharply defined and varies from a pale yellow colour to silver-white.

Cucumis melo (melon), *C. anguria* (gherkin), and *C. maderaspatanus* have been infected. (The American strain was not tested on the two last named plants.) A yellow mottle is the most characteristic symptom. Gherkin fruits may be mottled and deformed but no infected melon plants were grown to the fruiting stage.

Bryonia dioica, common bryony, was infected and showed a yellow mottle.

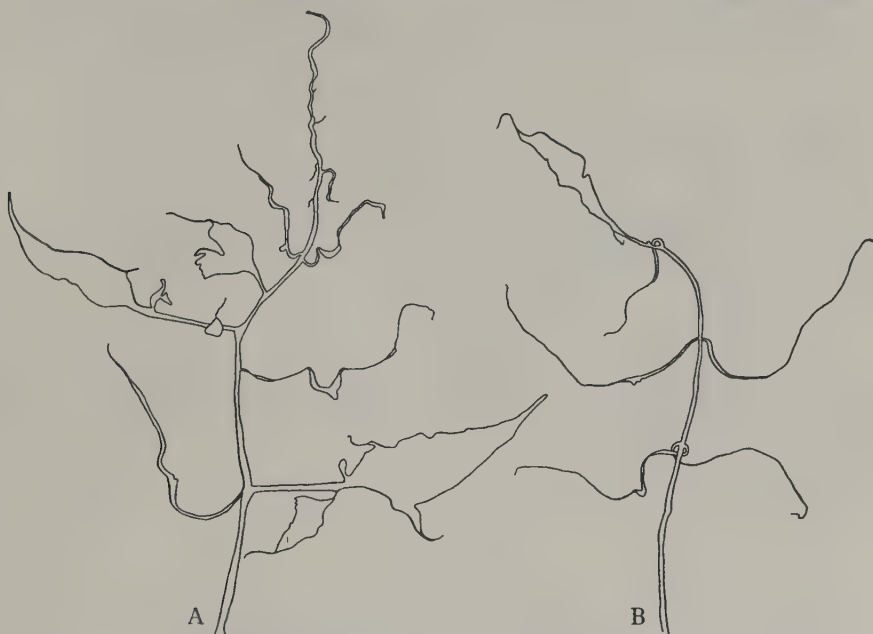
Citrullus vulgaris, water-melon (varieties Florida Favourite and Dixie). At different times 21 water-melon plants were inoculated but in no case did infection result.

Cucurbita pepo, vegetable marrow. A yellow mottle with some distortion of the leaves and stunting. The fruit may be mottled and deformed but few fruit usually develop on an infected plant. Ogilvie⁽¹⁶⁾, in 1928, was the first to record a mosaic of vegetable marrow in England the symptoms of which agree with those noted above. Mr L. Ogilvie kindly afforded me the opportunity to examine some of his diseased marrow material from the West of England. The virus appeared to be very similar to the cucumber strains in physical properties and in the symptoms produced on cucumber, tobacco and *Datura Stramonium*, but it differed in one respect by infecting water-melon (*Citrullus vulgaris*). Under the conditions of experiment systemic infection of water-melon (varieties Florida Favourite and Dixie) seedlings invariably occurred. Occasional diffuse pale yellowish spots developed on the leaves inoculated and systemic symptoms took the form of few to many rather inconspicuous pale greenish-yellow spots, sometimes with a small necrotic centre, on the leaves. Walker⁽²³⁾ described a natural outbreak of water-melon mosaic in Florida the symptoms of which were very much more severe than any obtained with the marrow mosaic virus.

Lycopersicum esculentum, tomato, is fairly readily infected but there is considerable variation in the symptoms. A very mild mottle without distortion or with slight narrowing of the leaves are the most usual symptoms, but on occasion leaf distortion becomes extreme to give the condition known as "fern leaf." A plant exhibiting typical fern leaf has the laminae deeply cut or reduced to such an extent that the leaflets become thread-like (see Text-fig. 1 *A* and *B*) and in severe cases the petals also are filiform. The occurrence of fern leaf on experimental inoculation (mechanical methods only were used) has been sporadic but independent

of the season. This agrees with the findings of Mogendorff⁽¹⁵⁾ who made a detailed study of fern leaf. He was, however, able to produce fern leaf at will by using the insect *Myzus persicae* as vector and he also showed that air temperature effects the type of symptom, the minimum, optimum and maximum air temperatures for the expression of fern leaf being 15° C., 18–22° C. and 25° C. respectively.

The fern leaf symptom like many symptoms produced by a virus on tomato is not absolutely diagnostic of the virus. Under conditions of high



Text-fig. 1. *A* and *B*. Tracings of tomato leaves distorted by *cucumber virus 1* (fern leaf).

light intensity *cucumber virus 1* is the only agent known to the writer able to produce an infectious fern leaf, but when light intensity is low, as in winter, *tobacco virus 1* causes considerable distortion of the foliage of young plants (see Text-fig. 2 *D, E* and Mogendorff⁽¹⁵⁾, *b*, p. 28) which is liable to be confused with true fern leaf. It is not usually difficult to distinguish the two conditions. If *tobacco virus 1* is the cause, the distortion is never so extreme as it can be when due to *cucumber virus 1*; and if the plant is subjected to better lighting conditions, leaves showing little or no distortion are produced and finally the plant has a crown of almost normal leaves with a fringe of filiform leaves below. A plant with true fern leaf

continues to produce filiform leaves under the improved conditions. The distortion due to tomato mosaic virus is very often called "fern leaf" but it would be better if this term was reserved for infection with *cucumber virus 1*.

Kraybill *et al.* (14) claim to have obtained a leaf-deforming principle, very resistant to heat (not inactivated by 126° C. for 2½ hours) in filtrates from mosaic diseased tomato plants, which is able to produce non-infectious filiform symptoms in leaves of young tomato plants on heavy inoculation. Unsuccessful attempts were made in the summer of 1932 and again in the spring of 1933 to obtain such a principle from tomato



Text-fig. 2. C. Healthy tomato leaf. D and E. Tomato leaves distorted by *tobacco virus 1*.

mosaic, yellow ("aucuba") tomato mosaic, single-virus streak and yellow-mottle mosaic of cucumber infected tomato plants. The last named showed fern leaf symptoms. Seven different samples of infected juice were experimented with but a non-infectious filtrate from fermented juice (except that infected with *cucumber virus 1* which ages rapidly) could not be obtained with the finest filters used (Jena fritted glass No. 4 and Pasteur-Chamberland L 7), while heating the filtrates to 98° C. for 10 min. always rendered them inactive.

When combined with potato mosaic virus *cucumber virus 1* gives streak symptoms in tomato (see also Valleau and Johnson⁽²²⁾) but the streak produced has not been so severe as a tomato mosaic-potato mosaic streak.

Nicotiana tabacum, tobacco (variety White Burley). Pale green circular spots may appear on the leaves inoculated two or three days after inoculation but no necrotic lesions are produced. Systemic infection first shows as a clearing of the veins and later a mild general mottle results. There is sometimes distortion of the leaves (narrowing) but the symptoms on tobacco may be very mild. The American strain of the virus was slightly more virulent on tobacco than the English. E. M. Johnson⁽¹¹⁾ distinguished three types of cucumber mosaic differentiated by the reactions of tobacco. The different isolations of the virus studied have produced no more variation in symptoms than that consistent with varying environmental conditions.

Nicotiana glutinosa. Mild to moderately severe mottle, slight narrowing and sometimes a little necrosis, of the leaves. Stunting of the plant.

Datura Stramonium. Faint pale green circular spots on the leaves inoculated, vein-clearing followed by a mild mottle and slight stunting.

Solanum nigrum is one of the commonest solanaceous weeds of glass-houses but attempts to infect this plant have failed.

Properties of cucumber virus 1.

*Filterability*¹. Will not pass Pasteur-Chamberland filters.

Resistance to ageing in vitro. Three days or less².

Resistance to heat. Usually completely inactivated by 60° C. for 10 min.

Resistance to alcohol (C₂H₅.OH). Inactivated by 50 per cent. alcohol in 1 hour.

INSECT VECTORS.

Cucumber virus 1 has a number of insect vectors. Under field conditions in America it is stated to be spread by the cucumber beetles *Diabrotica vittata* and *D. duodecimpunctata* (Doolittle^(5, 6)), and the following aphids have been reported capable of transmitting the disease: *Myzus persicae*⁽⁸⁾, *M. pseudosolani*, *M. circumflexus*, *Macrosiphum gei* (*M. solanifolii*)⁽⁹⁾ and the melon aphid, *Aphis gossypii*^(5, 6). Ogilvie and Mulligan⁽¹⁷⁾ suspected *Macrosiphum gei* to be a vector of marrow mosaic.

All the aphids occur in this country and the first four have been described and figured recently by K. M. Smith⁽²¹⁾. *A. gossypii* is found damaging cucumbers under glass and is thought to be the most likely vector in this country. *M. pseudosolani* and *M. circumflexus* are occa-

¹ On two occasions the virus was filtered but this result could not be repeated.

² The virus was found to be active on the third day twice in eight tests made at different times.

sionally found breeding on tomatoes under glass and *M. persicae* also occurs occasionally on tomatoes.

No insect vectors of *cucumber virus* 3 and *cucumber virus* 4 are at present known.

DISCUSSION.

The virus, or group of closely allied viruses, of which Johnson's *cucumber virus* 1 (12) is the type, is widely recognised and the yellow-mottle mosaic virus certainly belongs to this group. The marrow mosaic strain differs from the type in being able to infect water-melon (*Citrullus vulgaris*) and in this respect agrees with *cucumber virus* 2 described by Porter (19). *Cucumber virus* 2 is stated to differ from *cucumber virus* 1 in having an incubation period one to two days longer, giving slightly different symptoms on cucumber and by infecting Chinese Long cucumber, another plant resistant to *cucumber virus* 1. *Cucumber virus* 2 was filtered through a fritted glass filter (size <7) unchanged but no indication is given by Porter of its host range. It apparently ages rapidly as dry leaves were no longer infectious. At present it is impossible to say whether the marrow-mosaic virus should be identified with *cucumber virus* 2 or not. Another virus able to infect water-melon is *celery virus* 1 (Wellman (24)) but this virus is more resistant to heat (inactivated at 75° C.) and ageing (6-7 days) than the marrow-mosaic strain and gives local lesions only on *Datura Stramonium*.

Cucumber viruses 3 and 4 belong to the same group but differ from the *cucumber virus* 1 type in physical properties and host range (see above). A cucumber plant infected with *cucumber virus* 3, as already mentioned, nearly always exhibits a few yellow spots on the leaves, but further cucumber plants inoculated from such leaves reproduce the typical green-mottle symptoms. If, however, the centre is punched from one of the yellow areas and used as inoculum, it is found that a virus of the *cucumber virus* 4 type has been isolated. This result has a parallel in the work of Jensen (10) who obtained several strains of yellow viruses from *tobacco virus* 1 in a similar way. Price (20), also, obtained new strains of *cucumber virus* 1 by this method. A few preliminary experiments have shown that though a plant infected with either cucumber virus 3 or 4 can subsequently be inoculated with *cucumber virus* 1 and show symptoms due to the last named virus, a plant infected with *cucumber virus* 3 is with difficulty infected with *cucumber virus* 4 and *vice versa*. These results support the view that cucumber viruses 3 and 4 are related types and emphasise their distinctness from *cucumber virus* 1.

CONTROL MEASURES.

Bewley and Corbett⁽⁴⁾ brought forward evidence that cucumber mosaics were seed transmitted, and their recommendations for the use of clean seed have proved of value in reducing the incidence of the diseases. The percentage of seeds giving rise to diseased seedlings is low. Kendrick⁽¹³⁾ found that cucurbit mosaic (*cucumber virus 1*) was transmitted through musk-melon seed. He raised 11,519 plants (representing 23 packets of commercial seed) and obtained 27 mosaic diseased seedlings. The maximum transmission in one batch was 2.13 per cent. Ogilvie and Mulligan⁽¹⁸⁾ obtained one diseased plant from 256 seeds from mosaic diseased marrow fruit, and the writer obtained three diseased gherkin plants from 685 seeds from a commercial stock of seed suspected of being infected with *cucumber virus 1*.

The three mosaics described can be readily spread by juice inoculations so that the eradication or isolation of plants or blocks of plants as soon as symptoms are noticed and care in pruning reduces the rate of spread.

Weeds likely to act as alternate hosts should be destroyed. *Cucumber virus 1* is able to infect solanaceous weeds. The only common cucurbitaceous weed is *Bryonia dioica* (common bryony). It is highly probable that *cucumber virus 1*, at least, occurs naturally on this plant though the writer has been unsuccessful in transmitting any virus from wild bryony to cucumber in spite of repeated attempts from suspected plants.

Aphids, if present, should be controlled by fumigation.

SUMMARY.

Three cucumber mosaic diseases of this country and the causal viruses are described.

The first disease, green-mottle mosaic (*cucumber virus 3*), and the second, yellow mosaic (*cucumber virus 4*), are not transmissible to solanaceous plants. *Cucumber virus 3*, on cucumber, causes a dark green mottle with blistering and distortion of the leaves, but the fruit is not usually marked; while *cucumber virus 4* gives rise to a distinct type of leaf-mottle, yellow to silver-white in colour, and the fruit may be seriously marked. The third disease, yellow-mottle mosaic (*cucumber virus 1*), is characterised by a diffuse yellow mottle of cucumber leaves and fruit and is transmissible to solanaceous plants. Cucumber viruses 3 and 4 are described for the first time.

Notes are given on fern leaf of tomato, insect vectors and control measures.

I wish to express my indebtedness to Mr E. R. Speyer for notes on the occurrence of the aphids mentioned, to Mr O. B. Orchard for material and observations in nurseries, and thank Dr G. H. Pethybridge and Dr W. F. Bewley for help and advice.

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Fig. 1.



Fig. 2.



Fig. 3.

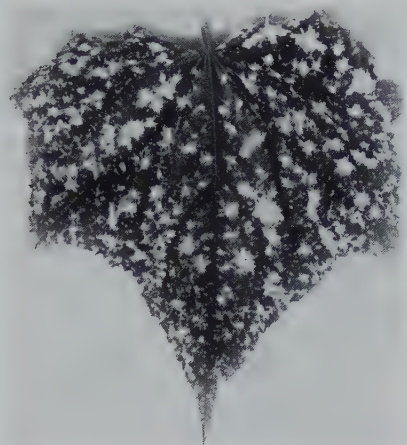


Fig. 4.



Fig. 5.



Fig. 7.

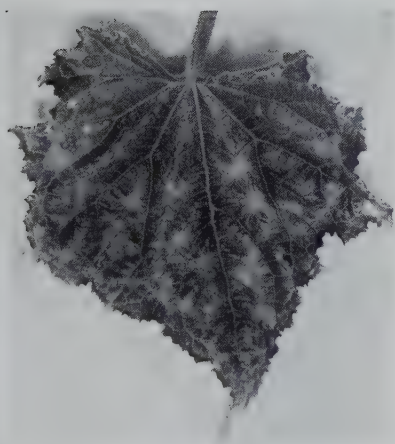


Fig. 6.



Fig. 8.



Fig. 9.



Fig. 10.



Fig. 11.



Fig. 12.

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EXPLANATION OF PLATES VI-VIII.

PLATE VI.

Green-mottle mosaic.

- Fig. 1. Cucumber, var. Butcher's Disease Resister, plant infected with *cucumber virus 3*. Summer symptoms.
 Fig. 2. Cucumber, var. Butcher's Disease Resister, plant infected with *cucumber virus 3*. Winter symptoms.
 Fig. 3. Water-melon, var. Florida Favourite, leaf showing symptoms due to *cucumber virus 3*. (Cf. Plates VII and VIII, figs. 8 and 12.)

PLATE VII.

Yellow mosaic.

- Figs. 4 and 5. Cucumber leaves showing yellow patterns produced by *cucumber virus 4*. (Cf. Plate VIII, fig. 9.)
 Fig. 6. Cucumber leaf showing winter symptoms due to *cucumber virus 4*. (Cf. Plate VI, figs. 1 and 2.)
 Fig. 7. Cucumber fruit marked by *cucumber virus 4*.
 Fig. 8. Water-melon leaf showing symptoms due to *cucumber virus 4*.

PLATE VIII.

Yellow-mottle mosaic.

- Fig. 9. Cucumber leaf showing mottle caused by *cucumber virus 1*. (Cf. Plate VII, figs. 4 and 5.)
 Fig. 10. *Bryonia alba* (white bryony) leaf from a plant naturally infected with *cucumber virus 1*.
 Fig. 11. Cucumber fruit showing mottle caused by *cucumber virus 1*.
 Fig. 12. Healthy leaf of water-melon.

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THE PHYSIOLOGY OF VIRUS DISEASES IN PLANTS

VII. EXPERIMENTS ON THE PURIFICATION OF THE VIRUS OF YELLOW MOSAIC OF TOMATO

BY JOHN CALDWELL.

*(Department of Plant Pathology, Rothamsted
Experimental Station, Harpenden.)*

(With Plates IX and X.)

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INTRODUCTION.

THE study of the nature of the virus, its chemical composition and its structure, has been rendered more difficult by the presence of plant-products in infectious juice. Various attempts have been made, therefore, from time to time, to obtain the virus agent in a medium containing as little extraneous material as possible; only by this means will any detailed study of the agent be possible. Any protein precipitant may be used to remove most if not all of the virus from a juice but, of course, much more than the virus is removed. Two main considerations have to be kept in mind. The precipitant must not itself destroy the virus, nor must it be so toxic to the plant that inoculation subsequently might prevent the entry of the virus into the host tissues by reason of the

destruction of the cells round the place of inoculation. Alcohol has commonly been used as a precipitant as many virus agents are resistant to exposure to 60 per cent. or so of alcohol. Beijerinck(3) showed that the alcohol precipitate from juice of mosaic tobacco plants could be separated and dried at 40° C. without much loss of infectivity. Allard(1) and others have shown that active material could be separated from diseased tobacco juice by talc, and by aluminium hydroxide. Brewer and Kraybill(4) adsorbed the virus on charcoal and so obtained an active product. Vinson(16) showed that acetone at 0° C. gave a precipitate with virus juice. Acetone, at a concentration of 60 per cent., had already been shown by Allard not to be toxic to the virus. The acetone precipitate was highly infectious. Saturation with ammonium sulphate also gives a highly infectious precipitate. In the same note Vinson states that a solution of safranin-O causes a quantitative precipitation of the viruses from the juice; in later papers he considerably modifies this statement. Vinson and Petre(17) report that in electrodialysis the virus of tobacco mosaic is found at the negative pole—a highly surprising result which is hardly consistent with their other observations. They found that basic dyes, *e.g.* Bismarck brown and safranin, precipitated the virus and that the virus could be freed from the precipitate by treatment with picric acid and sodium carbonate, or with amyl alcohol. They also found that basic lead acetate caused a heavy precipitate from infectious juice and that the virus was eluted by alkaline phosphate solutions whereas acid phosphate solutions did not remove it. They concluded that it is “probable that the virus we have investigated reacted as a chemical substance.” Woods(19) and Freiberg(9) had suggested previously that the virus was enzymatic in nature. In a later paper(18) Vinson and Petre gave more details of their method of precipitation and elution of the virus, and report that norite and talc added to a virus juice apparently increase the degree of infectivity. The estimates they make of the amount of virus present depend on the infection of numbers of tobacco plants, and, in the main, the percentage of infection among the control plants inoculated with untreated juice seems surprisingly low. In a recent note Barton-Wright and McBain(2) claim that by using Vinson and Petre’s technique they have been able to increase the purity of the virus extract enormously. Their work is discussed later in this paper.

In the animal virus field, the position is similar. Numerous workers, particularly Kligler and Olitsky(10), and Pirie(14), Kreuger and Tamada(11) and others, have been able to remove the infective principle from a serum. So far no one has succeeded in removing the virus from a plant juice into

a protein-free medium. Clearly, the higher the degree of purity which is obtained, the more precise will be our knowledge of the chemical and physical properties of the virus and to this end much work has been, and will in the future have to be, applied. At the present moment, only Barton-Wright and McBain⁽²⁾ for tobacco virus and Bronfenbrenner⁽⁵⁾ for bacteriophage have suggested that the virus might be carbohydrate in nature.

This paper gives some account of experiments which have been carried out with a view to the purification of the virus and of observations made on the purified material.

MATERIALS AND METHODS.

For work on the purification of the virus it is clear that only viruses which have a fair resistance to storage *in vitro* and to chemical treatment are suitable. The virus of yellow or aucuba mosaic of tomato is particularly well suited for this type of work, and was used in most of these experiments. It was obtained from its host-tomato (*Lycopersicon esculentum* var. "Kondine Red") for the most part, but occasionally it was obtained from tobacco (*Nicotiana tabacum* var. "White Burley"). The symptoms of the diseases caused by this virus have been fully described before⁽¹⁵⁾. It induces systemic mosaic on both tobacco and tomato, and a local necrosis on the leaves of *N. glutinosa*. *N. glutinosa* was used as a test plant for the virus since it has been shown in an earlier paper of this series⁽¹⁾ that the number of lesions on the leaf was proportional to the concentration of virus in any infective juice.

In the preparation of the juice in all the experiments the same technique was adopted. This has been described in an earlier paper⁽⁶⁾ and consisted of crushing the leaves with twice their weight of water. This material was kept overnight at room temperature to allow of the breakdown of the tissues and of the settling out of inorganic salts of various kinds. Next day, the macerated material was filtered through muslin and a greenish juice obtained. The chloroplasts and cell debris were easily removed by filtration through filter paper slightly impregnated with fuller's earth. This material was in the work recorded in earlier papers of this series considered as the prepared juice and was shown to contain much active virus. In this paper material of this kind is designated the prepared juice.

It has been shown that the virus will persist in an active condition in this material for many years, and that chemical treatment has relatively little effect upon it.

At each stage in the experiments outlined below tests were made by inoculation into *N. glutinosa* leaves to ascertain the amount of active virus present after the preceding treatment.

PRELIMINARY EXPERIMENTS.

A slight modification of the methods of Vinson and Petre was made to facilitate the preparation of as large a quantity as possible of the virus agent. The procedure adopted was as follows. To the prepared juice were added 5 percent. of a solution of basic lead acetate prepared by the standard method. A heavy precipitate—creamy coloured when tobacco juice and brown when tomato juice was used—was thrown down. This precipitate which was removed on a centrifuge was found to contain all the virus. The supernatant liquid was normally quite free from virus particles, though occasionally one or two spots would appear on the inoculated *glutinosa* leaves. The absence of necrosis following inoculation was not due to the toxicity to the plant tissues of the lead in the solution since no more infection was observed when the lead was precipitated by the addition of di-sodium-hydrogen phosphate to the liquid. Neither the precipitate so formed nor the supernatant liquid was found to contain virus.

The basic lead acetate precipitate was suspended in five or six times its own volumes of $M/3 \text{ KH}_2\text{PO}_4$ solution and left for two hours, being carefully shaken up from time to time. Thereafter the mixture was centrifuged to separate the precipitate. The supernatant liquid was found to be wholly free from virus or to have only a few particles present in it. The precipitate contained large quantities of virus. The process of suspension in acid phosphate and separation was repeated with the same result. Thereafter, the precipitate—now much less bulky—was suspended in a like quantity of $M/1 \text{ K}_2\text{HPO}_4$ solution and left overnight. After 24 hours the mixture was centrifuged, when the supernatant liquid was made up with distilled water to the original volume of the prepared juice. This liquid was found to contain practically as much virus as the original juice; some virus did, however, remain in the precipitate as was shown by suspending it in water and using it as an inoculum. The treatment of this material and the results obtained in a series of experiments are shown in Table I.

It was found that a second suspension and separation in $M/3 \text{ K}_2\text{HPO}_4$ was usually sufficient to remove the virus from the precipitate, in which case the second K_2HPO_4 precipitate on suspension in water was found to contain practically no virus at all.

Table I.

The preparation of the purified virus material.

Treatment	pH	Virus content (Necrotic spots on leaves of <i>N. glutinosa</i>)
(1) 40 gm. of infected leaf—80 c.c. water macerated and left overnight, strained through muslin and filtered through fuller's earth gave 100 c.c. juice	Approx. 5.79	Many spots
(2) 100 c.c. juice—5 c.c. basic lead acetate solution supernatant	Approx. 7.5	No spots
(3) Precipitate of (2)—20 c.c. $M/3 \text{ KH}_2\text{PO}_4$ supernatant made up to 100 c.c.	Approx. 4.8	Few spots
(4) Precipitate of (3)—20 c.c. $M/3 \text{ KH}_2\text{PO}_4$ supernatant made up to 100 c.c.	Approx. 4.6	Few spots
(5) Precipitate of (4)—20 c.c. $M/1 \text{ K}_2\text{HPO}_4$ supernatant made up to 100 c.c.	Approx. 7.35	Many spots
(6) Precipitate of (5)— H_2O	Approx. 7.30	Some spots

It was also found that the preliminary precipitation with basic lead acetate suggested by Vinson and Petre was under our conditions undesirable since a concentration of basic lead acetate as low as 3 per cent., while it did not remove all the precipitable material from the prepared juice, did remove in most cases all the virus, the supernatant liquid being inactive on inoculation into *N. glutinosa*.

When a sufficient amount of a 20 per cent. normal lead acetate solution to cause complete precipitation was added to the treated juice it was found that the supernatant liquid contained no virus. This liquid had a pH of 5.5 and gave a heavy precipitate on the addition of basic lead acetate.

In all the experiments recorded here, therefore, there was no preliminary precipitation with lead acetate but the single precipitation with basic lead acetate, as described, was adopted.

The use of H_2S to remove the lead from basic lead acetate precipitate supernatant was tried but was found unsatisfactory, as shown by Vinson and Petre, since the treatment made the suspension very alkaline, and the results were very irregular.

Other protein precipitants were also tried such as ammonium sulphate, mercuric sulphate, hydrochloric acid and trichloroacetic acid, but their use was discontinued because of the difficulty of inoculation after their use. As has been pointed out above, only these treatments which neither destroy the virus nor are themselves toxic to the plant tissues are of any value in work on the precipitation of the virus.

THE ELUTION OF THE VIRUS FROM THE BASIC LEAD
ACETATE PRECIPITATE.

It is clear that the hydrogen-ion concentration is the major factor in the elution of the virus from the basic lead acetate precipitate as described above. Alkaline potassium-hydrogen phosphate removed the virus from the precipitate while acid potassium-hydrogen phosphate did not. A series of buffer solutions of mixed potassium-hydrogen phosphates were made up so that they had pH values of approximately:

4.5 (KH_2PO_4) 5.0 5.5 6.0 6.5 7.0 8.0 (K_2HPO_4).

The basic lead acetate precipitate was prepared in the usual way and then was suspended in each of the solutions in turn for one hour, being well shaken. After each suspension the mixture was centrifuged and the supernatant liquid treated for the presence of virus. It was found that the solutions of 4.5 and 5.0 pH had little effect on adsorption of the virus on the precipitate, that a little virus was washed off at pH 5.5 and that at the higher pH values the virus was washed off with ease.

THE EFFECT OF pH ON THE VIRUS.

Since the pH of the solution is so important a factor in the removal of the virus from the precipitate, the effect of the pH of this solution on the virus was examined. A large quantity of $M/10$ K_2HPO_4 eluate was prepared as described above. This eluate, as we have seen, has a pH of approximately 7.0–8.0 and is highly infectious when inoculated. The alteration of the pH of this material may throw down a precipitate but when this took place the whole of the material—precipitate and liquid alike—was used as an inoculum. The pH of the prepared liquid was altered by the addition of KOH solution to increase the alkalinity or of H_3PO_4 to increase the acidity. In this way it was possible to alter the pH over a wide range without the addition of ions other than the original potassium, hydrogen, or phosphate which were present in the first instance.

As a preliminary study, the KH_2PO_4 eluate of the basic lead acetate precipitate was tested and found on inoculation to induce the formation of one or two spots on each leaf. The pH of this eluate was 4.4 and it was rendered more alkaline by the addition of KOH —making a pH of 6.4. At this pH further inoculations on the leaves of *N. glutinosa* were made but no increase in the number of spots was observed. The pH of the KH_2PO_4 eluate was, therefore, not responsible for the non-infection of the *N. glutinosa* leaves.

The K_2HPO_4 eluate which was used for the major portion of the experiments had a $p\text{H}$ of 7.4. This was inoculated into a series of leaves and caused a large number of spots on each leaf. To this eluate was added H_3PO_4 until the $p\text{H}$ was 2.2. It was put on one side for six hours. At this $p\text{H}$ the liquid was cloudy, and was well shaken before inoculation. The effect on the leaf-tissues was severe and the leaves were rather burnt by the acid. Numerous necrotic lesions typical of the virus were, however, formed. It was clear that the exposure of the virus for six hours to a $p\text{H}$ of 2.2 had little effect on its activity. This was confirmed by the inoculation of the same material after the $p\text{H}$ had been adjusted to neutral. To another portion of the eluate was added H_3PO_4 to give a $p\text{H}$ of approximately 3.0. This liquid was again cloudy, and, after being left on one side for 6 hours, was found to be as infectious as the original control. There was no burning of leaves in this instance.

The alkalinity of the eluate was increased by the addition of KOH . After six hours, at a $p\text{H}$ of approximately 11.0 the solution was quite clear and no virus activity was demonstrable on inoculation. The leaves were not burnt in this instance. The activity was restored to a considerable extent on the return of the $p\text{H}$ of the juice to 6.4 by the addition of H_3PO_4 . A similar exposure of the material to a $p\text{H}$ of approximately 9.8 had no obvious effect on the virus activity either before or after neutralisation.

The $p\text{H}$ of yet another portion of the eluate was adjusted to 4.7—the $p\text{H}$ (approximately) of the KH_2PO_4 eluate. This had the effect of making cloudy the material which was inoculated on to the leaves in the usual manner. This treatment did not reduce the virus content of the liquid.

THE AMOUNT OF NITROGEN IN THE PHOSPHATE ELUATES.

It was clear that the liquid obtained by elution of the basic lead acetate precipitate in the different phosphate solutions contained protein and that in the K_2HPO_4 eluate, in which the virus was recovered almost quantitatively, there was considerable protein material. In addition there was a large amount of pigment, the removal of which presented some difficulties which have not yet been overcome. Estimations of the amount of nitrogen present at the different stages in the treatment were made and the results of two representative experiments are given below. In all the experiments the nitrogen content was reduced in the K_2HPO_4 eluate to 5–10 per cent. of that of the original juice.

It will be seen, from the data in Table II, that one-half of the nitrogen present in the prepared juice is removed in the basic lead acetate super-

natant and that, of the rest, only a portion is washed off the precipitate when the virus is eluted with K_2HPO_4 solution. It is probable that the nitrogen removed in the basic lead acetate supernatant represents inorganic and other forms of non-protein nitrogen so that a higher proportion of the original protein is recovered in the K_2HPO_4 eluate than would appear from the figures for nitrogen.

Table II.

The amounts of nitrogen present in the treated materials at different stages.

Material	pH		Nitrogen (total mg.)	
	Exp. A	Exp. B	Exp. A	Exp. B
Juice	—	5.41	320.0	562.9
Basic lead acetate super.	—	5.96	173.5	344.2
KH_2PO_4 eluate	—	4.5	48.5	23.2
K_2HPO_4 super. eluate	—	6.7	37.2	26.0
Basic lead acetate ppt.	—	—	35.8	—

THE SEPARATION OF THE VIRUS FROM THE PROTEIN.

The data recorded above show that the virus may easily be recovered, practically undiminished in quantity, in a liquid, and in the presence of a much reduced protein content from that of the prepared juice. The next step was the removal of the virus from the protein, if that were possible, or the demonstration of the nature of the virus by either direct or indirect means. A series of methods of protein precipitations were tried and in each case the protein and the virus tended to be found together. Numerous experimenters have found this, particularly with tobacco mosaic, which by reason of its resistance to ordinary treatment is very suitable for experimentation. The most recent work is that of Barton-Wright and McBain, to which allusion has been made. They found that the reconversion of the mixed phosphates in the viruliferous eluate of Vinson and Petre to KH_2PO_4 and the addition of two volumes of acetone to one of the acidified eluate resulted in the formation of a precipitate. This precipitate they found in their experiments to be largely protein. There was nothing new in the precipitation of the protein by acetone and they merely confirmed the findings of other workers that the protein took the virus down with it. The supernatant liquid in similar experiments done in this laboratory was always free of virus. The acetone present seems to have no serious effect on the leaves of *N. glutinosa* on inoculation.

Barton-Wright and McBain, however, went on to a separation of the precipitate into a colloidal (the protein) and a crystalline fraction. This

crystalline fraction, they allege, was typical of virus juice and was not found when healthy juice had been treated in exactly the same way as the virus material. They, therefore, by implication, make it appear that the virus is in a highly purified state in these crystals. Exception has already been taken to this position by the present writer (8), who has been unable to obtain crystals which are at once nitrogen-free and virus-containing. The methods and results are outlined below.

The K_2HPO_4 eluate was prepared in the usual way and was found to have a pH of 7.8. To this was added sufficient H_3PO_4 to give a pH of 4.7. This material was slightly cloudy. To it was added two volumes of acetone when a heavy precipitate was thrown down. This precipitate on examination was found to consist of two portions—a crystalline portion and a colloidal. The supernatant was free from virus and both the crystalline and the colloidal portions appeared to contain virus. There was comparatively little virus in the crystalline material but a great deal in the colloidal portion. The colloidal precipitate can readily be shown to be protein.

Up to this point the writer is in agreement with Barton-Wright and McBain. The constitution of the crystals is the point in which the differences occur. Briefly, they find that no crystals are formed when healthy juice is treated as was the infected, and that the crystals, though they contain no nitrogen, were found to cause infection on inoculation into tobacco. That is to say, they contained some virus—the amount of which is unknown.

The writer's experience has been as follows. The crystalline portion of the precipitate from infective juice was washed in acetone and water mixture (2 acetone to 1 of water by volume) and, so far as possible, all the colloidal material removed. Thereafter the crystals were washed once or twice with acetone, which was decanted off and then they were dried. These crystals differ slightly if prepared from tomato or tobacco tissue. Those from tomato are slightly brown in colour as against the white crystals from the tobacco tissue. It was found that these crystals in aqueous solution contained a small trace of virus—the tests for the presence of virus were made on *N. glutinosa* leaves and were, therefore, quantitative as well as qualitative. A little of this aqueous solution was tested serologically for the presence of protein and was found to give positive results. Similar crystals were also examined microanalytically and were found to contain a trace of organic material which charred with sulphuric acid and also enough nitrogen to make possible a certain demonstration of its presence. The serological tests were made possible

by the kindness of Dr J. M. Birkeland and the microanalyses were carried out by my colleague Mr F. J. Richards. When similar crystals were dissolved in water and reprecipitated it was found that the supernatant liquid contained a trace of virus and the resulting crystals contained less virus than the original crystals.

The microscopic examination of the crystals showed that in outline they resembled closely the crystals of KH_2PO_4 . For this reason, similar experiments were set up with healthy juice and with the reagents themselves in the absence of juice.

The healthy material on similar treatment produced crystals identical in appearance with those of the infectious juice. It is therefore evident that the presence of virus in a juice had no direct connection with the appearance of the crystals.

The addition of two volumes of acetone to one of an aqueous solution of KH_2PO_4 causes the precipitation of crystalline material, the amount increasing with the concentration of phosphate in the solution. An $M/15$ solution of KH_2PO_4 contains approximately 9.0 gm. of salt per litre. 50 c.c. contains therefore 0.45 gm. The addition of 100 c.c. (two volumes) of acetone to 50 c.c. of this solution results in the formation of dense white precipitate of needle-shaped crystals (not a faint opalescence as suggested by Barton-Wright and McBain). After half an hour there is a deposition of the crystals at the bottom of the container. At ordinary room temperature the yield of crystals is approximately 0.3 gm. from 50 c.c. of solution. The first reaction in this and in the earlier cases is the formation of bulky precipitate of needle-shaped crystals which disappear as the liquid is allowed to dry up, *e.g.* on a microscope slide, and are replaced by the larger rhombic crystals shown in the figures (Plates IX and X, figs. 1-3). The rhombic crystals have the same shape in the presence or absence of either healthy or infectious plant juice. It is clear, therefore, that the crystals are formed by the action of the only substances common to all three groups of materials, *viz.* the reagents.

The microscopical examination of the crystals during and after formation reveal some very important details. It was noticed that in the experiments involving plant juice, either healthy or infected, the needle-shaped crystals which are formed immediately on the addition of the acetone to the acidified eluate tend to be aggregated, quite irregularly, into groups round colloidal material. In these same experiments an examination of the final crystals showed that they had apparently been formed round masses of colloidal material. It is suggested that the effect of acetone on the eluate at a pH of 4-5 is to precipitate the protein and

the crystalline KH_2PO_4 simultaneously so that the colloidal material acts as a nucleus for crystal formation. The figures in Plate X illustrate the appearance of the crystals which are obtained from diseased juice, healthy juice and from the reagents in the absence of protein material. It is clear from them that the colloidal material which is present in the first two types of crystals and absent from those from the reagents alone make difficult the separation of the crystals from organic material after precipitation from an acetone-phosphate mixture.

The observation that the crystals of phosphate contained colloidal material led to the setting up of another group of experiments. In the earlier experiments the concentration of the phosphate used in elution was $M/10$. It was found, by experience, that more concentrated phosphate solutions had the effect of damaging the tissues of the leaves of *N. glutinosa* on inoculation and, therefore, were unsatisfactory in quantitative virus studies. For the purposes of precipitation, however, as has been pointed out, the higher the concentration of phosphate the greater the amount of the crystalline portion of the precipitate. A series of K_2HPO_4 solutions were prepared with concentrations of $M/10$, $M/5$, and $M/1$. These were used in the elution of the different basic lead acetate precipitated from both healthy and infected juice. Thereafter, they were acidified with H_3PO_4 to $p\text{H } 4.5$ —which, in effect, entails the conversion of the K_2HPO_4 to KH_2PO_4 —and two volumes of acetone added. It was found that with the $M/10$ eluate the greater portion of the precipitate was colloidal in nature with a small crystalline portion, that practically all the precipitate was crystalline in the case of $M/5$ K_2HPO_4 eluate, and that there was a large crystalline precipitate in the $M/1$ K_2HPO_4 eluate. In other words, the colloid material was used as the nucleus for crystal formation and, when the quantity of phosphate was sufficiently large, the protein material was all included in the crystals.

THE NITROGEN CONTENT OF THE PURIFIED CRYSTALS.

In the foregoing sections the question of the precipitation of the protein and the salts by acetone has been discussed. Some further details of the crystalline portion of the precipitate are now to be considered. The reaction immediately following on the addition of acetone to the phosphate eluate is the formation of a heavy precipitate of mixed colloid and crystalline nature—the proportions varying with the concentration of phosphate in the eluate. When $M/10$ phosphate is used only small amounts of crystals are obtained. For the purposes of these experiments involving chemical analysis, therefore, $M/5$ K_2HPO_4 solutions were used. After

elution and acidification two volumes of acetone were added. The precipitate was readily separated by decantation and the crystalline portion separated from the colloidal. The supernatant was put into a refrigerator at -5°C . As a consequence, after a few days a further precipitate was formed. This precipitate appeared to be wholly crystalline on examination and it was thought that this probably contained little of the colloid material. On inoculation into *N. glutinosa* the crystals were found to contain traces of virus, and microanalysis demonstrated in them the presence of organic material and of traces of nitrogen.

A series of microanalyses on the purified crystals were carried out. As a control KH_2PO_4 crystals were precipitated from an *M*/1 aqueous solution with acetone and were collected. These were found to contain no demonstrable nitrogen on analysis. Similar crystals were prepared from the phosphate eluate of precipitates from both healthy and virus juice and these were found to contain in the unpurified state between 0.1–0.2 per cent. of nitrogen. Actually, the difference in nitrogen content between the crystals from healthy materials and those from diseased tissue was not significant. Even those crystals which were obtained by keeping the supernatant in a refrigerator for some days—and crystals did form under these conditions—were found to contain slight traces of nitrogen.

THE PRECIPITATION OF THE PROTEIN IN THE JUICE.

In a series of experiments on the juice of infected plants results were obtained which are germane to this study. Juice prepared by filtration through fuller's earth was used in these experiments. To a portion of this juice was added two volumes of acetone. A heavy precipitate was obtained and this was removed on a centrifuge. The precipitate was made up to the original volume with water and was found to contain all the virus, the supernatant liquid being almost completely virus-free. *N. glutinosa* was used as the test-plant. A second portion of the juice was heated to 75°C . for 15 min. and a precipitate was formed. This precipitate was also centrifuged off and made up with water to the original volume. In this instance, both the precipitate and the supernatant were found to contain virus. A third portion of the juice was put into a U-tube the ends of which were closed with cellophane held in position by rubber bands. The tube was inverted so that each arm was under water in a separate beaker. Platinum electrodes were inserted into the beakers and a current of 100 volts D.C. was passed through the liquid. At the end of two hours a large precipitate had formed in the U-tube and the water in the beakers contained some quantity of electrolytes. The water in the

beakers was changed frequently and the current allowed to run for twelve hours. At the end of that time the precipitate was separated from the supernatant liquid, made up to the original volume with water, and both were inoculated into *N. glutinosa* leaves. The precipitate contained much virus, the supernatant induced the formation of only a few spots on inoculation.

To a fourth portion of the juice was added sufficient ammonium sulphate to give a saturated solution. A dark precipitate was thrown down which was separated from the supernatant by centrifugation and made up with water to the volume of the juice originally saturated. This material contained much virus. The supernatant was electrodyalysed as above described, and no precipitate was formed nor was any virus found to be present on inoculation.

Yet another portion of the juice was saturated with magnesium sulphate, and the precipitate removed. This precipitate contained virus. The supernatant was electrodyalysed, when a second precipitate was formed which, on inoculation, was also found to contain virus. The second supernatant was virus-free. The same juice was then diluted one in ten with water and various substances added to different portions. Liquid soap and various enzymes were used. The juice was kept exposed to the action of these substances for 24 hours at room temperature, after which inoculations were made. The results obtained are recorded in Table III.

Table III.

Effect of soap and enzymes on infectivity of juice.

Treatment	No. of spots	Treatment	No. of spots
Control juice	∞	0.25 % Taka diastase	3-6
1/500 Liquid soap	∞	2.5 % Pepsin	20
1/200 Liquid soap	1-4	2.5 % Trypsin	0-1
1/100 Liquid soap	0	2.5 % Papain	0
2.5 % Taka diastase	0-1	0.25 % Papain	0-1

Thereafter the juice to which the enzyme had been added was heated to 75° C. for 15 min. and further inoculations made. The results are recorded in Table IV.

Table IV.

Effect of heating enzyme-juice mixture at 75° C. for 15 min.

Treatment	No. of spots
Control	∞
2.5 % Taka diastase	0-1
2.5 % Pepsin	10-20
2.5 % Trypsin	∞
2.5 % Papain	0

When juice which had been exposed to liquid soap at a concentration of one in five was acidified with phosphoric acid after 72 hours' treatment a white precipitate was formed. The mixture was shaken up and inoculated into *N. glutinosa* when, despite the slight burning of the leaves, numerous spots were formed.

DISCUSSION.

From the results detailed above it is probable that any protein precipitant will remove the virus of yellow mosaic of tomato from infected juice. Whether the virus can be recovered from the precipitate or not depends on the action of the precipitants. The virus is active over a very wide range of pH —from approximately 2.5 to 10.5—and outside that range the virus is not necessarily destroyed by the acidity or alkalinity since, as has been pointed out, the neutralisation of juices of pH above and below the extremes of this range resulted in a return of the virus symptoms on inoculation. Whether, in these instances, the excessive acidity or alkalinity had temporarily inactivated the virus or whether the effect of the acidity or alkalinity was to prevent the entry of the agent into the plant is a point difficult to settle, but the weight of the evidence is in favour of the latter explanation. It would appear that, were the virus even in an inactive state, to gain entry into a broken cell the pH of the cell would restore the activity lost by reason of the excessive acidity or alkalinity of the medium.

It cannot be too emphatically stated that the effect of the reagents used on the actual cells of the host plant, *e.g. N. glutinosa*, is probably the main factor in the non-development of symptoms after many of the treatments to which the virus juice has been subjected. Many times it has been found that reagents which produced no actual necrosis of the leaf-tissues were effective in preventing the development of typical lesions.

From the experiments on precipitation, recorded in this and in other papers, it may be concluded that the virus is either protein in nature or is so closely adsorbed to the protein that, on any alteration of the physical state of the juice, the virus reacts as does the protein. This holds for all viruses of the tobacco mosaic group, at least, and for many others in other groups.

The results of the experiments with enzymes are of interest in this connection. Various workers have at different times recorded experiments with enzymes and, since the virus does react like a protein in so many ways, the interest of the reaction of proteolytic enzymes on the

virus is obvious. In a recent paper Lojkin and Vinson⁽¹²⁾ report the results of some experiments on the effect of enzymes on the virus of ordinary tobacco mosaic (Johnson's tobacco virus no. 1). It is of interest to note that they give no precise data regarding the concentration of the enzyme they used, although they report that some enzymes would inactivate "purified" virus but not virus in crude juice. It might well be that the concentration of the virus in the crude juice was such as to be greater than the enzyme present could inactivate, though this is improbable. They found that under the conditions of their experiment, emulsin, pepsin and yeast extract did not reduce the infectivity of the virus juice. They conclude further, that the "inactivation of the virus by enzymes is not due to adsorption—it seems likely that the inactivating effect of the enzyme solution is due to its hydrolytic effects." The present writer's results bear out some of the conclusions of Lojkin and Vinson. He has found repeatedly that pepsin even in such high concentrations as 2.5 per cent. is unable completely to inactivate the virus of yellow mosaic of tomato and that after 24 hours' incubation with 2.5 per cent. pepsin a large proportion of the virus is unaffected, as judged by the formation of necrotic lesions on the leaves of *N. glutinosa*. At lower concentrations of pepsin there is no evidence of any effect on the concentration of the virus. Under similar conditions, however, trypsin has the effect of apparently completely inactivating the same virus in 24 hours at room temperature. This, as has been pointed out before (Caldwell⁽⁷⁾), does not mean the destruction of the virus since heating at 75° C. for 15 min. restores the greater portion of the virus activity. Whether the effect of the trypsin is to inactivate the virus, which is reactivated on heating, or whether it is to prevent the adsorption of the virus by the cell walls is difficult to determine. 2.5 per cent. papain, on the other hand, inactivated the virus in 24 hours and no recovery was observed after treatment at 75° C. for 15 min., which is not effective in destroying the proteolytic activity of the enzyme. The difficulty of removing the enzyme, or at least inactivating it, has occasioned a good deal of work on this material. The general conclusion to be drawn from a large number of experiments seems to be that the enzyme may act on the broken tissue of the inoculated leaves and tend to prevent the entry of the virus into the tissues rather than that it destroys the virus itself. The available evidence of many experiments is summarised in Table V. In some experiments advantage was taken of the facts that iodine and HgCl_2 are supposed to act as inhibitors and that the optimum pH for papain activity is about 5.0 with just below 4 and above 9 as extremes (see Oppenheim⁽¹³⁾).

Table V.
The effect of papain on the virus.

Conc. of papain %	Treatment	pH	No. of spots
0.5	Immediate inoculation	—	0
0.5	Inoculation after 24 hours	—	0
0.5	Boiled before addition to virus	—	Many
0.5	Inoculation after treatment at 75° C. for 10 min. following 24 hours' incubation	—	1-2
1.0	Inoculation after 24 hours	—	0-1
1.0	Inoculation after treatment at 75° C. for 30 min. following 24 hours' incubation	—	Few spots
1.0	Washed off immediately after inoculation after incubation	—	0-1
1.0	Washed off immediately after inoculation without incubation	—	0
1.0	Immediate inoculation	5.3	0-4
1.0	Inoculation after 72 hours' incubation	5.3	2-4
1.0	+ KOH and exposed 24 hours	9.21	1-2
0.1	Exposed 24 hours	5.72	Many
0.1	" "	4.24	"
0.2	" "	3.14	"
0.2	" "	9.23	Some spots
0.2	" "	5.58	Many

From these experimental results it appears that a high concentration of papain is necessary to destroy or prevent the activity of this virus. Low concentrations are ineffective in preventing the formation of necrotic lesions on inoculated leaves of *N. glutinosa*. It has not yet been clearly established that the effect of the papain is on the actual virus and not on the tissues of the inoculated leaves, and work on this aspect of the problem is being continued.

SUMMARY.

Experiments on the purification of the virus of yellow mosaic of tomato are described and discussed. Vinson and Petre's methods of purification of the virus from infectious juice and subsequent elution with phosphate solution were slightly modified in these experiments. It was found that there was no evidence that the virus could be recovered in a crystalline form and that viruliferous material always contained traces of organic nitrogen. This virus was found to be active over a wide range of pH, viz. from 2.0 to 10.5. At the extremes of the scale, the excessive acidity or alkalinity was toxic to the inoculated leaves and adjustments had to be made before inoculation. Different protein precipitants were used in an attempt to free the virus from the proteins, and electrolytic

methods were also tried. Proteolytic enzymes were employed on the purified virus juice but the results were rather unsatisfactory. Difficulty was experienced in ensuring that the effect of some reagents was on the virus and not on the tissues of the test plants.

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Fig. 1.

CALDWELL.—THE PHYSIOLOGY OF VIRUS DISEASES IN PLANTS (pp. 68-85).



Fig. 2.



Fig. 3.

EXPLANATION OF PLATES IX AND X.

PLATE IX.

Fig. 1. Leaves of *Nicotiana glutinosa* plants inoculated with (1) treated juice; (2) basic lead acetate supernatant; (3) first KH_2PO_4 eluate; (4) second KH_2PO_4 eluate; (5) first K_2HPO_4 eluate; (6) second K_2HPO_4 eluate; (7) third K_2HPO_4 eluate; (8) precipitate + water. For details see text.

PLATE X.

Fig. 2. Crystals from $M/15$ KH_2PO_4 solution with virus or healthy juice added (precipitated with acetone).

Fig. 3. Crystals from $M/15$ KH_2PO_4 solution, precipitated with acetone.

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ON THE GALL MIDGES INJURIOUS TO THE CULTIVATION OF WILLOWS

II. THE SO-CALLED "SHOT HOLE" GALL MIDGES (*RHABDOPHAGA* SPP.)

BY H. F. BARNES, M.A., PH.D.

(*Entomology Department, Rothamsted Experimental Station.*)

(With Plates XI-XIV and 3 Text-figures.)

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I. INTRODUCTION.

THIS is the second of a series of papers⁽¹⁾ on the gall midges which are injurious to cultivated willows. In this contribution it is proposed to deal with the group whose larvae live in the stems or rods resulting in the so-called "shot hole" damage. The "shot holes" are in reality nothing more than the exit holes prepared by the larvae and completed by the pupae in the stems of the willows for the emergence of the adult midges.

As is usual in work of this nature, the writer is indebted to several persons for supplying material. It is a pleasure to express thanks in this connection to Messrs H. P. Hutchinson, T. Justin Cowley, W. E. H. Hodson, N. B. Warner Bromley and Sir Stephen Tallents.

Where dates of occurrences in the life history of these midges are mentioned, it must be understood that they refer to Harpenden under cold greenhouse and insectary conditions unless specifically stated otherwise.

It is to be hoped that this paper will demonstrate clearly the great value to systematists of a knowledge of the bionomics of a species of insect as well as of the morphological characters of the adult.

II. IDENTIFICATION.

One of the first mentions of a "shot hole" gall midge of willows was in 1841 when Léon Dufour described *Lasioptera saliciperda* as a new species. This species has since been placed in the genus *Rhabdophaga* Westwood. His description is not really adequate for the differentiation of this midge from other closely allied species, but he did mention the large elongated pointed process at the base of the antennal sheath in the pupa. The only reference to its host plant that he gave was the trunk of a living willow. However, after a careful investigation of the literature the writer considers that the species of midge found on *Salix fragilis* and *S. caerulea* is almost certainly the same species, and accordingly the name *R. saliciperda* Dufour is retained for it.

In 1861 Giraud mentioned that he had frequently found midges doing damage of this nature to willows, among others *S. purpurea*, and described in detail a similar midge from *Populus alba*. He apparently was under the impression that only one species of midge was involved in this type of damage (viz. "shot holes") and wrote of them as *Cecidomyia saliciperda* Dufour. However, Kieffer later renamed Giraud's midge from poplar as *R. giraudiana* Kieffer (1898).

In 1847 Westwood described a midge doing similar damage to *S. viminalis* and *S. rubra* as *Cecidomyia (Rhabdophaga) viminalis* sp.n. The writer considers that this name should be retained in case a gall midge of this group is found later to occur on *S. viminalis*. Up to the present he has not been successful in finding one.

Since these early times several other midges have been described from the stems of *Salix* spp. These may be divided into those whose larvae produce a distinct or marked swelling on the stems, e.g. *R. salicis* Schr. (1803), *R. dubiosa* Kieff. (1913) [new name for *dubia* Kieff. (1892)] and *R. karschi* Kieff. (1892), and those that produce no

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swelling or only a slight one, e.g. occasionally *R. karschi* Kieff. (1892), *R. saliciperda* Duf. (1841), *R. viminalis* Westw. (1847), *R. medullaris* Kieff. (1892), *R. pierrei* Kieff. (1896) and *R. nielsenii* Kieff. (1906). There are other species which are closely associated with the buds on the stems, e.g. *R. pulvini* Kieff. (1891), *R. gemmicola* Kieff. (1896), *R. superna* Kieff. (1897), *R. insignis* Kieff. (1906) and *R. perforans* Kieff. (1906). In the case of *R. gemmicola* and *R. superna* the larvae live actually in the bud and need not be further considered here. Of the remaining three, the adult of *R. pulvini* is recorded as emerging through the bud, while those of *R. insignis* and *R. perforans* emerge through the pad at the base of the bud or petiole.

In all these species the emergence holes of the adults may be likened to shot holes and thus could be included in the term "shot hole" midges. However, this term is usually limited to those midges whose larvae live in the stems of willows without causing any marked swelling, i.e. those in Table I.

For the sake of clearness it is useful to tabulate the midges, including those to be described in this paper, whose larvae live in the stems of willows without producing a marked swelling. This is done in Table I, the host plants and the number of antennal segments originally mentioned for each species being also given. This latter character has been proved⁽²⁾ to be variable but it serves as an indication of what may be expected.

Table I.

Species of gall midges whose larvae do not form a marked gall or swelling, but live in the stems of willows, i.e. "shot hole" midges.

Midge	Host plant originally mentioned	Number of antennal segments originally mentioned
<i>R. saliciperda</i> Dufour (1841)	"Saulé vivant"	"15-articulatis"
<i>R. viminalis</i> Westwood (1847)	<i>S. viminalis</i> , <i>S. rubra</i>	"17 joints"
<i>R. karschi</i> Kieffer (1892)	<i>S. aurita</i> , <i>S. cinerea</i>	♂, 2 + 16; ♀, 2 + 15
<i>R. medullaris</i> Kieffer (1892)	<i>S. aurita</i>	♀, 2 + 14 - 15
<i>R. pierrei</i> Kieffer (1896)	None originally given, later <i>S. aurita</i> and <i>S. cinerea</i>	Not mentioned
<i>R. nielsenii</i> Kieffer (1906)	<i>Salix</i> spp.	2 + 15

The following species are dealt with in this paper:

Midge	Host plants	Antennal segments
<i>R. saliciperda</i> Dufour (1841)	<i>S. caerulea</i> , <i>S. fragilis</i>	♂, 2 + 14 (99. 1) 13* ♀, 2 + 14 (93. 7) 13
<i>R. triandraperda</i> sp.n.	<i>S. triandra</i>	♂, 2 + 17 (53. 45. 2) 15 ♀, 2 + 17 (13. 73. 14) 15
<i>R. purpureaperda</i> sp.n.	<i>S. purpurea</i>	♂, 2 + 16 (7. 75. 18) 14 ♀, 2 + 16 (42. 57. 0. 1) 13
<i>R. justini</i> sp.n.	<i>S. purpurea</i>	♂, 2 + 15 (35. 65) 14 ♀, 2 + 15 (9. 91) 14

* The method of determining this antennal formula is described in *Proc. zool. Soc. Lond. for 1932*, pp. 323-34.

Kieffer stated in his monograph⁽⁴⁾ that *R. saliciperda* Dufour is found on narrow-leaved willows, while *R. pierrei* Kieffer is found on willows with large and hairy leaves (*Salix aurita*, *cinerea* and *caprea*).

The present writer while dealing with cultivated species of willows¹ has found four distinct species of gall midges producing "shot hole" damage. One of them has been identified as *R. saliciperda* Dufour but the other three are reluctantly described as new species. It has been experimentally proved that these species are each definitely restricted to one species of *Salix*, while *R. saliciperda* Dufour uses two species as host plant. For this reason, until the wild species of *Salix* have been studied, it is at least unwise to associate the midges at present under consideration, and about which a good deal is known as regards bionomics and recognition characters, with midges described rather inadequately from wild species of willows. Furthermore, specimens of these species have not been available for study.

From a perusal of the literature it is obvious that in many instances, when "shot hole" midge damage has been found on willows, the midge has been assumed to be *R. saliciperda* Dufour without due examination of the insect itself and without the species of willow being considered, in fact merely on the presence of "shot holes." As a result of such erroneous records one reads in standard reference books that the host plants of *R. saliciperda* are *S. alba*, *aurita*, *caprea*, *cinerea*, *fragilis*, *helix*, *nigra*, *purpurea*, *triandra*, *viminialis*, *viridis*. Such diversity of host plants is in direct contrast to the experience of the present writer. The same is true for the other previously described "shot hole" midges, but in a less degree, owing probably to the fact that their names were less well known to the enthusiastic recorders of anything new, such as new host plants.

III. *RHABDOPHAGA SALICIPERDA* DUFOUR.

This midge was originally described by Dufour in 1841 and a valuable paper concerning it⁽³⁾ was written in 1912 by Dr Giacomo Cecconi. It is evident both from the letterpress and photographs that he was dealing with this species, but unfortunately he enumerated a long list of host plants based largely on old erroneous records. His list reads: "*S. alba*, *aurita*, *caprea*, *cinerea*, *fragilis*, *nigra*, *purpurea*, *triandra*, *viminialis*, *viridis*," and he even mentions an old record of the species on *Populus alba*. From his work however emerges the fact that *R. saliciperda* Dufour does attack *S. alba*.

¹ *Salix alba*, *alba* var. *vitellina*, *americana*, *caerulea*, *nigricans*, *purpurea*, *purpurea* × *viminialis*, *repens*, *triandra*, *triandra* × *viminialis* and *viminialis*, with *S. fragilis* in addition.

(a) *Description.*

Naked eye appearance when alive is suggestive of a *Lasioptera* species rather than of a typical *Rhabdophaga* species.

Male. Length about 2-2½ mm., heavily built. *Antennae:* formula (0·7) 2+14 (99. 1) 13, each flagellar segment (except the last) consisting of basal enlargement and neck; length and breadth of neck on 3rd flagellar segment as 2 : 1·75, length of basal enlargement two and a half times as long as neck; length and breadth of neck on 10th flagellar segment as 2 : 1, length of basal enlargement two and a half times as long as neck. *Palps:* thickly clothed with scales, three distal segments about equal in length and breadth, almost quadrate, slightly longer than proximal segment, shorter than in the other three species to be described. *Thorax and abdomen:* dark brown, covered with long hairs and with broad, almost black, scales dorsally on abdomen. *Wings:* opalescent, whitish, strongly reminiscent of *Semudobia betulae* Winn. in general appearance, posterior portion of wing very delicate and faint, 5th vein almost obsolete, wing densely covered with hairs, anterior margin with black scales and hairs, posterior margin with fringe of long hairs, which are easily lost in mounting. *Legs:* silvery in dried specimens, tarsi clothed with silvery scales; claws with only faint basal tooth, empodium well developed, as long as claws. *Genitalia:* basal clasp segment short, rather stout; terminal clasp segment stout; dorsal lamella bilobed, each lobe rather long, medium width, rounded; ventral lamella deeply emarginate, each lobe moderately narrow, blunted; harpes dark, moderate length, with digitiform processes distally; style stout.

Cecid. 1713-15 and 2303-6 reared from *S. fragilis*.

Cecid. 2285-9 and 2292-3 reared from *S. caerulea*.

Female (Plate XI, fig. 1). Length 2½-3 mm. *Antennae:* formula (1·0) 2+14 (93. 7) 13, each flagellar segment elongated bead-like, with neck practically absent or at the most very short and transverse. *Wings:* as in male opalescent, whitish. General appearance of body dark, almost black, on closer examination deep red with black bands of scales dorsally. *Ovipositor* pocket-shaped. Otherwise about as in male.

Cecid. 1716-17, 2301-2 and 2307-9 from *S. fragilis*.

Cecid. 1856-7, 2290-1 and 2294-8 from *S. caerulea*.

Pupa. When empty, head parts pale brown. Antennal sheaths with large elongated pointed process at base (Text-fig. 1, A).

Cecid. 1708-9 and 2299-300 from *S. fragilis*.

Cecid. 1851, 1855, 2130 and 2284 from *S. caerulea*.

Larva. Anchor process broad, well chitinated, head of anchor rather variable, slightly to well emarginated, centre portions more heavily chitinated (Text-fig. 3, A and B).

Cecid. 1632-3 from *S. fragilis*.

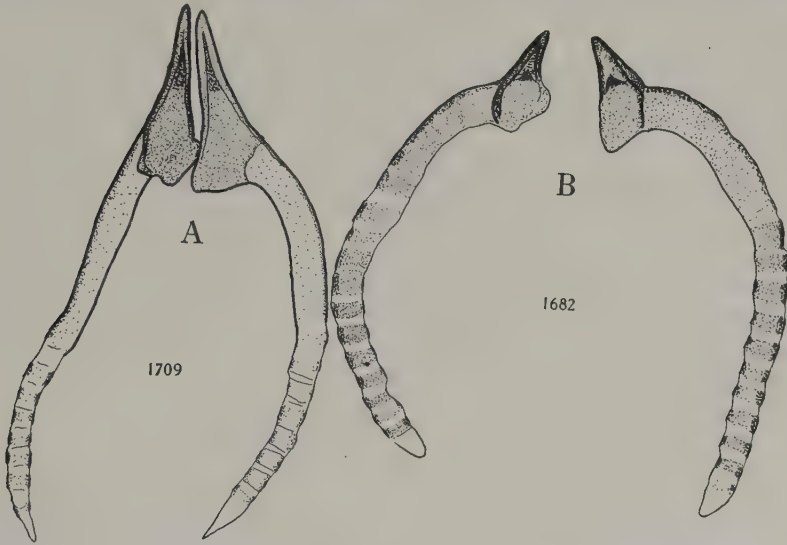
Cecid. 1849-50 and 1938-9 from *S. caerulea*.

Habitat. *S. fragilis* and *S. caerulea* (Cricket Bat Willow). In old branches, and first and second year twigs in cricket bat willow nurseries. Berkshire and Kent.

(b) *Biology.*

This species is single brooded. The adult midges emerge from late April until early July. For example, specimens bred on *S. fragilis* emerged from May 23rd to July 2nd in 1931, and from May 4th until May 31st in 1933. Specimens reared on *S. caerulea* emerged from April 30th to May 21st in 1932 and from April 16th to May 24th in 1933.

The adults can be at once distinguished from any of the three following midges owing to the opalescent whiteness of their wings reminiscent of those of *Semudobia betulae* Winn. Their egg-laying habits,



Text-fig. 1. Empty antennal sheaths from pupae of A, *R. saliciperda* Dufour;
B, *R. triandraperda* sp.n. $\times 60$.

however, are very similar to the other shot hole midges. The eggs are laid both on the stubs and branches. When under test with newly planted sets they oviposit on the set and the fresh young green shoots as well. Usually there is no swelling, but when several larval cavities or chambers occur close together on a small shoot it has a slightly swollen appearance. The larvae live in clusters each in its own chamber as do the next two species. Birds rip up the bark in their search for the larvae. After successful emergence of the midges there are shot holes visible as in the case of the other species.

Individuals reared on Bat willow (*Salix caerulea*) and Crack willow (*S. fragilis*) from different localities have been successfully mated and

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the progeny of these pairings have been reared on both kinds of willows. Similarly midges of *S. caerulea* parentage have been bred on *S. fragilis* and *vice versa*. Thus there is no doubt that only one species is involved. The slight discrepancies in the emergence dates of midges on *S. fragilis* and *S. caerulea* indicated above are probably due to the fact that the material was collected in two localities during the late winter and early spring. By this time the larvae were full grown and had probably adjusted themselves to the weather conditions in their own localities. Their removal to Harpenden was too late for them to overcome this influence and emerge at the correct dates for the Harpenden area. The *S. caerulea* material came from Newbury, Berkshire, while the *S. fragilis* material was collected near Dartford, Kent. The insect was also found attacking a nursery of *S. caerulea* grown for sets near Dartford. In this case the midge was obviously spreading on to the *S. caerulea* from nearby Crack willows.

This midge is a species which reproduces by means of unisexual families as has been proved by rearing the progeny of isolated females.

The host plants of this species are *S. caerulea* and *S. fragilis* and also *S. alba* (Cecconi, *loc. cit.*). Emphasis must therefore be laid on the dangers attendant upon growing Bat willows in any neighbourhood where wild Crack willows are to be found. The insect has not been found on any other species of willow, but insectary tests have not yet been made.

IV. *RHABDOPHAGA TRIANDRAPERDA* SP.N.

(a) *Description.*

Male. Length $2\frac{1}{2}$ –3 mm., heavily built. *Antennae:* formula (1.0) 2+17 (53. 45. 2) 15, each flagellar segment (except the last) consisting of basal enlargement and neck; length and breadth of neck on 3rd flagellar segment as 3:2, length of basal enlargement slightly over twice as long as neck; length and breadth of neck on 10th flagellar segment as 3:1, length of basal enlargement nearly twice as long as neck. *Palps:* thickly clothed with long hairs and a scale or two, variable in size, frequently two proximal segments about equal, distal two segments longer and distinctly longer than broad. *Thorax and abdomen:* dark greyish brown, thickly clothed with long dark brown hairs, dark narrow scales dorsally on abdomen. *Wings:* densely covered with hairs, especially anterior margin, well-developed fringe of long hairs on posterior margin which is easily rubbed off. *Legs:* dark, thickly clothed with dark scales and hairs; claws bifid, empodium well developed, about as long as claws. *Genitalia:* basal clasp segment long, stout; terminal clasp segment short, stout; dorsal lamella bilobed, each lobe broad, rounded; ventral lamella deeply emarginate, each lobe long, narrow, points blunted (isosceles triangular); harpes slightly digitiform apically; style short, broad. Co-types: Cecid. 1110, 1122–4, 1131, 1133–7 and 1466.

Female (Plate XI, fig. 2). Length about $2\frac{3}{4}$ – $3\frac{1}{4}$ mm. *Antennae:* formula (1.0) 2+17 (13. 73. 14) 15, each flagellar segment elongated bead-like, with neck practi-

cally absent or at the most very short and transverse. General appearance of body dark red. *Ovipositor* pocket-shaped. Otherwise about as in male.

Co-types: Cecid. 1108, 1120-1, 1127-9, 1132 and 1467-8.

Pupa. When empty, head parts brown. Antennal sheaths with large triangular process at base (Text-fig. 1, B).

Cecid. 1109, 1111, 1125-6, 1130 and 1682-4.

Larva. Anchor process heavily chitinated, head of anchor bilobed and with 2 wing-like processes (Text-fig. 3, C), thus closely resembling that of *R. pierrei* Kieff. figured by Kieffer⁽⁴⁾ on Plate 31.

Cecid. 1086-7, 1451, 1930-3 and 2153.

Habitat. *S. triandra*, especially Black Maul variety. In stubs and bases of shoots. Suffolk and Leicestershire.

(b) *Biology*.

This species has one brood a year. The adult midges emerge from the middle of April until the beginning of June according to the season. For instance in 1928 emergence started on April 13th and continued until May 25th; in 1930 it commenced on April 24th and did not stop until June 5th.

Just before emergence, which takes place at a regular time of day and chiefly between 8 and 10 a.m. (standard time), the pupae protrude from the bark. As soon as the females are free of the pupal cases they are fertilised by the males which rush, fluttering their wings, madly up and down the willow shoot or stub. Before mating the females rest motionless, with their ovipositors extruded, close beside their own particular pupal case. But very soon after the act, they start crawling over the willow in a spider-like fashion. They keep on inserting their ovipositors into crevices and crannies until they find a satisfactory position, in which they lay one or more eggs in quick succession. As a rule, unless disturbed, the midges do not move far away from their pupal cases nor fly until they have got rid of a portion of their eggs. Oviposition goes on more or less continuously throughout the first 8-10 hours of the midges' existence by which time nearly all the eggs have been laid. Occasionally a female will go on laying on the second day after emergence. Virgin females have been kept alive for $5\frac{1}{2}$ days, but impregnated ones usually die within 48 hours of their emergence.

Besides ovipositing in crevices on the stub, this midge lays eggs at the insertion of leaves on shoots, at the insertion of shoots on the stock, in between axillary buds and the stem, and even on young stems and on the undersides of leaves which have not yet grown out from the apical buds. This is at any rate true on newly planted sets on which the bark is more smooth and free from crevices than on an old stub. The midge

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however is definitely thigmotropic as regards oviposition. In fact in experimental work it has been quite a routine practice to scar the bark of the willows in order to induce oviposition.

The eggs are bright red in colour. In size they appear to vary slightly with age; when extracted from the female after fertilisation they measure $33 \times 7\mu$, when 95 hours' old they are $36 \times 8\mu$ and when 125–192 hours' old they are $38 \times 10\mu$. (Freshly hatched larvae measure $34 \times 10\mu$.) The eggs took 10–13 days to hatch in 1928; for example, eggs laid on May 24th and 25th started hatching on June 3rd and all the larvae had appeared by June 6th. The number of eggs which can be laid by a single female is in the neighbourhood of 150. This figure was obtained by dissecting impregnated females prior to the commencement of oviposition. Those eggs which are laid on the bark are usually tucked away out of sight, whereas those laid in other positions are sometimes visible.

On hatching, the larvae soon disappear from sight and work their way into the tissue immediately below the skin of the willow. Here they lie, more or less bathed in sap, in small cavities or depressions usually parallel to the long axis of the plant. In these cavities they grow and remain throughout their larval life which extends over practically a year. In one instance eggs were laid on April 26th–27th, 1930, and the full grown larvae were extracted from the twigs the following April 26th and 27th. It would appear that the larvae grow during the season of plant growth and remain as fully grown but developing larvae throughout the autumn and winter. About a week or fortnight prior to the emergence of the midges, these larvae change into pupae.

Although it is usual to find colonies of larvae, each in its own cavity, sometimes isolated larvae occur. This follows naturally on the oviposition habits of the midges and the lack of movement of the larvae. As a rule the presence of larvae in a willow is not noticeable and no swelling of the twig is apparent, but there are two exceptions to this statement. Firstly, when many eggs have been laid close together on a young twig or shoot, a slight swelling results; secondly, birds, such as tits, peck at infested stubs and branches and so tear the bark into shreds, thus probably doing more damage to the plant than a slight infestation of midges. The resulting midges from any surviving larvae oviposit near the initial attack and so it is often possible to obtain midge material by using the birds' evidence of a previous attack. The easiest way of recognising an attack is by means of the exit holes of the pupae which just before emergence stick out of the willow stub or branches. The empty pupal cases remain in this position until they are blown away, leaving only the shot-like holes.

From some fifty attacked willow sets about 5–8 in. long, which had been discovered in the first place by birds, 524 midges were reared giving a sex ratio of $47\frac{1}{2} : 52\frac{1}{2}$. It was however noticed that from individual sets midges of one sex only sometimes emerged. A test for unisexual families, such as occur in *R. heterobia* H. Lw., and *R. terminalis* H. Lw., resulted in proving this midge does reproduce by means of unisexual families. It was further found possible to mate a single male with as many as twelve females. (Mating lasts on the average¹ about 23 seconds.) This enabled a test to be made as to whether the male was the controlling agent in the determination of the sex of the offspring. When six females were mated to a single male, each of them produced all-male families; but this result is inconclusive, owing to the small size of the families resulting from the different females, and the experiment was not repeated because of the difficulties experienced in getting the females to lay the full number of eggs.

The host plant of this species is *S. triandra*, especially the Black Maul variety. Unsuccessful efforts have been made to rear it on *S. purpurea* (Dicky Meadow), *S. viminalis* \times *purpurea* (Harrison) and Poplar. Eggs were laid on Harrison quite readily and to a less extent on Dicky Meadow and Poplar, but no larvae survived. It appears that egg laying depends nearly as much on the roughness or smoothness of the bark on the variety of plant as on the ability of the larvae to survive on it. The species has not been found in the field on any species of cultivated willow other than *S. triandra*. It has been found in Suffolk and in Leicestershire, in the latter county occurring both in the Newark and the Wanlip willow beds.

V. *RHABDOPHAGA PURPUREAPERDA* SP.N.

(a) *Description.*

Male. Length $2\frac{1}{2}$ –3 mm., heavily built. *Antennae:* formula (0.6) 2+16 (7. 75. 18) 14, each flagellar segment (except the last) consisting of basal enlargement and neck; length and breadth of neck on 3rd flagellar segment as 2 : 2, length of basal enlargement three times as long as neck; length and breadth of neck on 10th flagellar segment as 2 : 1.5, length of basal enlargement slightly less than three times as long as neck. *Palps:* with long hairs and a few scales, variable in size, usually third and distal segments about equal in length, second shorter, proximal segment still shorter, second, third and fourth segments distinctly longer than broad. *Thorax and abdomen:* greyish brown, with broad dark scales dorsally on abdomen which is slightly haired. *Wings:* broad, densely covered with hairs, anterior margin clothed with scales and long hairs, the fringe on the posterior margin of wing being easily rubbed off during the mounting of specimens. *Legs:* light straw colour in dried specimens, thickly clothed with dark

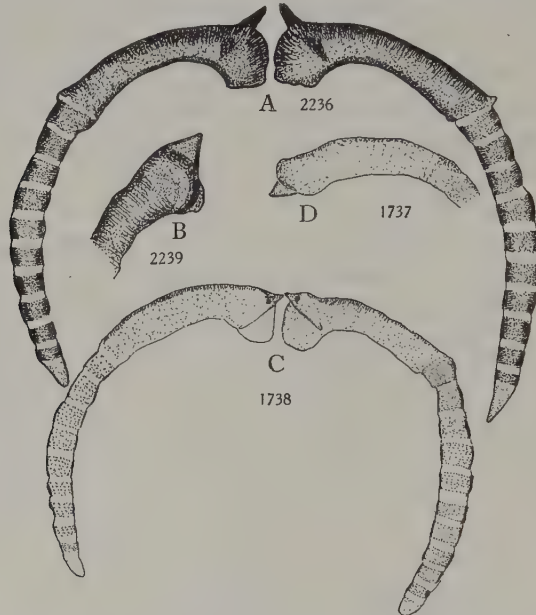
¹ Of nineteen pairings.

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scales and hairs; claws bifid, empodium well developed, about as long as claws. *Genitalia*: basal clasp segment stout; terminal clasp segment stout basally, gradually tapering; dorsal lamella bilobed, each lobe large, almost as broad as long, smoothly rounded; ventral lamella strongly emarginate, each lobe short, rounded (equilateral triangular); harpes, very dark, blunt ended, with several digitiform processes apically and dense hairs apicad; style short, broad.

Co-types: Cecid. 1694, 1853-4, 2232-5 and 2245-9.

Female (Plate XI, fig. 3). Length 3-4 mm. *Antennae*: formula (1.0) 2+16 (42. 57. 0.1) 13, each flagellar segment elongated bead-like, with neck practically absent or at the most very short and transverse. General appearance of body large



Text-fig. 2. Empty antennal sheaths from pupae of A, *R. purpureaperda* sp.n.; B, lateral view of same; C, *R. justini* sp.n.; D, lateral view of same. $\times 60$.

red, on closer examination red with black bands of scales dorsally. *Ovipositor* pocket-shaped. Otherwise about as in male.

Co-types: Cecid. 1692, 2240-4 and 2250-8.

Pupa. When empty, head parts almost black. Antennal sheaths with moderately large triangular process at base (Text-fig. 2, A and B).

Cecid. 1680-1, 1852 and 2236-9.

Larva. Anchor process well chitinised, head of anchor process bilobed with centre portion more heavily chitinised than the sides (Text-fig. 3, D).

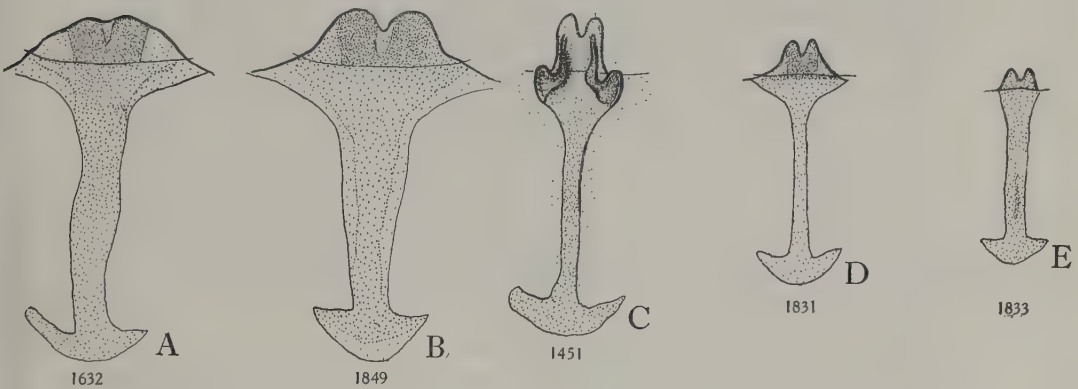
Cecid. 1830-1.

Habitat. *S. purpurea*, especially Dicky Meadow variety. At base of shoots. Lancashire.

(b) *Biology.*

This species has one brood a year. Emergence of the adult midges occurs from the middle of April until the beginning of July. For example, in 1931 midges started emerging on April 30th and continued until July 7th; in 1932 the dates were April 29th and July 7th; but in 1933 (an early year for midges) emergence started on April 6th and continued until June 15th.

The emergence, mating and oviposition of this species are very similar to those of the former species. So also are the eggs and the larval habits. Eggs in 1931 took 6–12 days to hatch. The midges usually lay their eggs low down on the young shoots both in captivity where newly



Text-fig. 3. Anchor process of larvae A, *R. saliciperda* Dufour from *S. fragilis*; B, the same from *S. caerulea*; C, *R. triandraperda* sp.n.; D, *R. purpureaperda* sp.n.; E, *R. justini* sp.n. $\times 100$.

planted sets have been used each year, and also in the field where each year's growth is cropped. Thus usually there are only the new young shoots and stubs available during the period of oviposition. If however the previous year's shoots are not cut down, the midge will oviposit high up the shoots on the 1-year-old wood and also on the new side shoots. The writer has never seen an attack actually on stubs in the field but in captivity the eggs are readily laid on the original set. The eggs however are more often left exposed than with the previous species which nearly always attempts to put them in crevices.

The life history too is very similar. Eggs which were laid on May 6th, 1931, developed into adult midges which started emerging on April 29th, 1932; similarly adult midges started emerging on April 6th, 1933, from eggs laid on May 8th–13th, 1932. The larvae are sometimes clustered

together and, as in *R. triandraperda*, they lie each in a separate cavity parallel to the long axis of the shoot. There is usually no swelling of the infested shoot. Birds recognise their presence and pick out the larvae. After emergence the same tell-tale shot holes are left to indicate the previous presence of the insect.

This is another species in which unisexual families occur. Three isolated families laid eggs in 1931 and the resulting families in 1932 consisted of 27 females, 34 males and 39 males respectively. In another set of experiments using single females (1932-3), families of 69 females, 51 females and 15 females were raised; in addition the use of two females resulted in a generation of 153 females. Although in these instances the eggs were nearly always laid on one day (sometimes two days), the resulting midges emerged over a period of from 35 to 71 days. The usual length of life of the adults is about 36 hours. Mating lasts on the average¹ 39 seconds, and the males will mate with more than one female. The egg stage lasts 6-12 days, the pupal stage about 14 days and the larval stage 10-11 months.

Attempts have been made to mate this species with individuals of *R. triandraperda*. In one case only did engagement take place and that was between a female of this species and a male of the former. Eggs were laid but were unfertile. No successful matings were accomplished in the reverse direction. Similarly attempts to cross this species with *R. heterobia* and *R. terminalis* were unsuccessful.

The host plant of this midge is *S. purpurea* (Dicky Meadow variety). It has not been found in the field on any other variety. Under cold greenhouse and insectary conditions attempts have been made to rear it on *S. triandra* (Black Maul), *S. caerulea*, *S. alba* var. *vitellina*, *S. alba*, *S. fragilis*, *S. viminalis* (Long Skin), *S. nigricans*, *S. repens* and *S. americana*. In no instance did larvae live although eggs were sometimes laid.

The species has been found in Lancashire.

VI. *RHABDOPHAGA JUSTINI*² SP.N.

(a) *Description.*

Male. Length about 2 mm., lightly built compared with previous three species. *Antennae:* formula (1.0) 2+15 (35. 65) 14, each flagellar segment (except the last) consisting of basal enlargement and neck; length and breadth of neck on 3rd flagellar segment as 3:1.5, length of basal enlargement twice as long as neck; length and breadth of neck on 10th flagellar segment about as 2.75:1, length of basal enlargement

¹ Of eight pairings.

² The writer has pleasure in naming this species in honour of his friend Mr T. Justin Cowley.

about twice as long as neck. *Palps*: with scales and a few long hairs, variable in size, usually proximal segment shortest, second, third and distal segments usually progressively longer. *Thorax* and *abdomen*: light greyish brown, thorax almost devoid of hairs, a few hairs on abdomen, broad dark brown scales dorsally on latter. *Wings*: narrow, densely covered with hairs, anterior margin clothed with scales and long hairs, posterior margin with fringe of long hairs which are easily lost in mounting. *Legs*: dirty silvery in dried specimens, clothed with scales and hairs; claws bifid, empodium well developed, about as long as claws. *Genitalia*: basal clasp segment moderate width; terminal clasp segment moderate width; dorsal lamella bilobed, each lobe broadly rounded; ventral lamella nearly U-shaped emargination, each lobe moderately short, blunted, narrow; harpes with conspicuous digitiform process distally; style short.

Co-types: Cecid. 1695, 2259-64 and 2267-71.

Female (Plate XI, fig. 4). Length about 2-2½ mm. *Antennae*: formula (0·7) 2+15 (9. 91) 14, each flagellar segment elongated bead-like, with neck practically absent or at the most very short and transverse. General appearance of body small, pink. *Ovipositor* pocket-shaped. Otherwise about as in male.

Co-types: Cecid. 1693, 1696 and 2272-81.

Pupa. When empty, head parts pale brown. Antennal sheaths with small triangular process at base (Text-fig. 2, C and D).

Cecid. 1737-8, 2265-6 and 2282-3.

Larva. Anchor process well chitinated, head of anchor bilobed, uniformly chitinated (Text-fig. 3, E).

Cecid. 1832-3.

Habitat. *S. purpurea*, especially Dicky Meadow variety. First brood at base of shoots, second brood higher up shoots and more scattered. Lancashire.

(b) *Biology*.

This species normally has two broods a year but occasionally there is a partial third flight. Adults of the overwintering generation emerge from the end of April to the beginning of June. In normal seasons, such as 1931, the second flight of midges occurs in July, but in an early year like 1933 midges of this generation emerge in the latter half of June. In such a year as this a partial third flight takes place during the first half of August. Some actual dates dealing with 1933 emergences are shown in Table II.

Table II.
Dates of emergences of R. justini sp.n., 1933.

Dates of oviposition of parent midge or midges	Dates of emergence of G_1 midges	Dates of emergence of G_2 midges
1. April 27	June 13-July 3	August 12
2. April 27-May 3	June 14-24	August 12-15
3. April 28	June 13-22	August 12
4. April 29-May 6	June 23-July 2	c. August 5
5. May 4-12	June 19-July 4	—
6. May 6-8	June 27	—
7. June 13-20*	c. August 5	—

* In this case the experiment began with midges of the 2nd 1932 flight instead of ones from the 1st 1933 flight (overwintering larvae 1932-3) as in the other examples.

The midges oviposit on any new growth that is available, but have not been observed laying on old wood. The eggs of the spring flight of midges therefore are laid low down on the current year's shoots, while those of the summer flying midges are deposited comparatively high up as a rule. The eggs, which are red and very similar to those of other *Rhabdophaga* species, are not usually laid in batches and so the larvae are more solitary and widely spaced than either *R. saliciperda* Dufour, *R. triandraperda* sp.n., or *R. purpureaperda* sp.n. However, they live in small cavities just as the other species do and shot holes are left after successful emergence, thus serving to indicate an attack. The larval chambers are to be found more or less anywhere on the stems in relation to the leaf bases and lateral buds, sometimes just below a bud, sometimes alongside and sometimes just below. Occasionally eggs are laid on or near the midvein of a leaf into which the larvae work their way and are able to develop successfully. In Plate XIV, fig. 2, an empty pupal case (B) can be seen sticking out of the midvein of a leaf on the right of the photograph. Fig. 1 on the same plate shows a shoot that has died as the result of several larvae living on it in close proximity. Normally there is no swelling of the stem as only one or two larvae are in close proximity, but a slight swelling occurs if several larvae are close together. An indication that the larvae are full grown and about to pupate is the appearance of small circular dark marks on the shoots due to the mature larvae preparing the exit holes for the pupae. The skin of the willow at these marks is very thin and, when the pupa is ready to protude, prior to the emergence of the midge, it is easily broken. One such dark patch or pre-emergence window can be seen at A in Plate XIV, fig. 2. These patches are most easily seen on the light green stems, and as the shoot grows old it becomes more difficult to see them; after the winter it is impossible to spot this evidence of a larval chamber. They are most useful in finding larvae of the first generation. After emergence has taken place the skin over the larval chamber discolours and often caves in, thus further indicating the previous presence of the larvae (Plate XIV, fig. 3, A). Birds are able to find these larvae and extract them with great skill. Two galls on this figure (B) have had the larvae extracted.

This species also reproduces by means of unisexual families as was proved by breeding from isolated females. In one instance a family of 99 females only was reared, thus indicating that females can lay about 100 eggs at least.

Thus six species of *Rhabdophaga* multiply in this way, viz. *R. heterobia* H. Lw., *R. terminalis* H. Lw., *R. saliciperda* Dufour, *R. triandraperda*

sp.n., *R. purpureaperda* sp.n. and *R. justini* sp.n. These are the only *Rhabdophaga* species that have been tested for this phenomenon so far.

All attempts to crossmate this species with *R. purpureaperda* sp.n. have failed.

The host plant is *S. purpurea* (Dicky Meadow variety). In addition, larvae of a midge most probably this species have been found once in the field on Mawdesley variety which is *S. purpurea* \times *viminalis*. Under experimental conditions repeated attempts have been made to rear the insect on *S. caerulea*, *S. fragilis*, *S. americana*, *S. alba*, *S. alba* var. *vitellina*, *S. triandra* (Black Maul), *S. nigricans*, *S. viminalis* (Long Skin), *S. repens* and *S. triandra* \times *viminalis* (Black Top), but in no case did larvae succeed in living on these plants.

This species has been found in Lancashire.

VII. DAMAGE AND CONTROL.

The damage caused by each of these four species of "shot hole" midges is very similar. The larvae all live in separate cavities just below the skin of the willow. In all four, the bark of the willows is ripped open by birds hunting for the larvae and so the actual insect damage is vastly increased. In the case of *R. triandraperda* sp.n., the larvae are confined to the stubs and bases of the shoots, the stub being thus weakened and the bottom foot of the rod often rendered useless both for cutting sets and for basket making. These types of damage are also typical of *R. purpureaperda* sp.n. Owing to *R. justini* sp.n. having more than one brood, the larval chambers are to be found right up the shoots and are not confined to the lower portions, weak points in the rods thus occurring throughout their length. *R. saliciperda* Dufour causes damage to the stubs, branches, and also to the current year's growth, and this species is decidedly injurious to Bat willow being grown for sets. The danger of wild Crack willows being allowed to grow in hedgerows is obvious and a promising Bat willow nursery could easily be ruined in this way. Damage to old shoots and branches is also often serious.

The control of these midges is difficult, partly owing to the fact that the damage is usually only noticed after birds have exposed their presence. Tarring the stubs has been suggested and this might be successful for *R. triandraperda* sp.n., *R. purpureaperda* sp.n. and *R. saliciperda* Dufour if the bases of all attacked shoots were burnt at the same time. With *R. justini* sp.n., the difficulty is the summer brood which, as stated before, is scattered all over the shoots and not limited to the base. In cases of serious infestations, if one season's entire crop of rods were to be

burnt, the destruction of the midge should be ensured. On the other hand, cutting the new growth down in May should reduce the midge numbers if the emergence and oviposition of the spring brood were nearly completed by the date of cutting. This method is actually practised in one district in order to get rid of initial caterpillar and frost damage to Dicky Meadows, the result being that the subsequent growth is straight and unbranched. This last method could be applied to all four midges providing the willows are able to recover from such a drastic set-back to their spring growth. The variety Dicky Meadow (*S. purpurea*) can recover sufficiently well to produce a good crop after this treatment.

Where Bat willows (*S. caerulea*) are grown, wild Crack willows (*S. fragilis*) should be ruthlessly cut down, because they serve as reservoirs for *R. saliciperda* Dufour as well as other Bat willow pests.

VIII. RECOGNITION CHARACTERS.

Keys have been constructed in order to facilitate the identification of these midges. Upon finding the typical "shot holes" on a cultivated willow, every effort should be made to identify the species of willow. If this is possible, the following key will prove helpful:

A. On *S. caerulea* (and on *S. fragilis*).....**R. saliciperda** Dufour.

AA. On *S. purpurea* (if the willow rods are cut annually).

B. "Shot holes" low down on stems**R. purpureaperda** sp.n.
and/or **R. justini** sp.n.

BB. "Shot holes" high up on stems.....**R. justini** sp.n.

AAA. On *S. triandra***R. triandraperda** sp.n.

The pupae (Text-figs. 1 and 2) can be distinguished as follows:

A. Head parts almost black.

B. Antennal sheaths with moderately large triangular process at base.....
R. purpureaperda sp.n.

AA. Head parts brown.

B. Antennal sheaths with large elongated pointed process at base.....
R. saliciperda Dufour.

BB. Antennal sheaths with large triangular process at base
R. triandraperda sp.n.

BBB. Antennal sheaths with small triangular process at base
R. justini sp.n.

The full grown larvae can be distinguished by an examination of the head of their anchor processes (see Text-fig. 3) as follows:

A. With wing-like processes **R. triandraperda** sp.n.

AA. Without such processes.

B. Large and broad**R. saliciperda** Dufour.

BB. Not so large or broad.

C. Centre portion more heavily chitinised than sides
R. purpureaperda sp.n.

CC. Small, uniformly chitinised.....**R. justini** sp.n.

The adults are much more difficult to recognise. The following characters, which have proved useful to the writer, are offered as an aid to identification in the field:

- A. Wings whitish opalescent **R. saliciperda** Dufour.
 AA. Wings not opalescent. General impression of bodies of females.
 B. Large dark **R. triandraperda** sp.n.
 BB. Large red **R. purpureaperda** sp.n.
 BBB. Small pink **R. justini** sp.n.

There do not appear to be characters for distinguishing the males without detailed examination under the microscope.

IX. PARASITES.

The writer is indebted to Dr Ferrière of the Imperial Institute of Entomology (through the courtesy of Sir Guy Marshall) for identifying some of the parasites that have been reared from these midges. His identifications together with the names of the host midges are given in Table III. *Tetrastichus roesellae* De Geer has been previously reared by the writer from *Dasyneura alopecuri* Reuter and *D. leguminicola* Lintner.

Table III.

*Hymenopterous parasites of R. saliciperda Dufour, R. triandraperda sp.n.,
 R. purpureaperda sp.n. and R. justini sp.n.*

Parasite	Host midge	Notes
Torymidae		
<i>Torymus</i> sp., near <i>auratus</i> Fonsc.	<i>R. triandraperda</i> sp.n.	Suffolk, 1930
Eurytomidae		
<i>Eurytoma aciculata</i> Ratz.	<i>R. triandraperda</i> sp.n.	Suffolk, 1930
<i>Eurytoma saliciperdae</i> Mayr.	<i>R. saliciperda</i> Dufour	Berkshire, 1932, on <i>S. caerulea</i> and Kent, 1931, on <i>S. fragilis</i>
Pteromalidae		
<i>Tridymus salicis</i> Nees	<i>R. triandraperda</i> sp.n. <i>R. saliciperda</i> Dufour	Suffolk, 1930 Kent, 1933, on <i>S. fragilis</i>
Eulophidae		
<i>Pleurotropis</i> ? <i>caenus</i> Walk.	<i>R. purpureaperda</i> sp.n. or <i>R. justini</i> sp.n.	Lancashire, 1931
<i>Tetrastichus flavovarius</i> Nees	<i>R. triandraperda</i> sp.n. <i>R. saliciperda</i> Dufour	Suffolk, 1930 Berkshire, 1932, on <i>S. caerulea</i>
<i>Tetrastichus roesellae</i> De Geer*	<i>R. triandraperda</i> sp.n. and <i>R. purpureaperda</i> sp.n. or <i>R. justini</i> sp.n.	Suffolk, 1930 Lancashire, 1931
Platygasteridae		
<i>Platygaster cecidomyiae</i> Ratz.	<i>R. saliciperda</i> Dufour	Kent, 1931, on <i>S. fragilis</i>
<i>Platygaster</i> sp. (? <i>philinna</i> Walk.)	<i>R. triandraperda</i> sp.n. and <i>R. purpureaperda</i> sp.n. or <i>R. justini</i> sp.n.	Suffolk, 1930 Lancashire, 1931

* As interpreted by Nees.

X. SUMMARY.

1. The species of gall midges whose larvae live in the stems of willows have been briefly reviewed.

2. It is pointed out that, in the past, the so-called "shot hole" midge damage on willow stems and branches has been frequently ascribed to *R. saliciperda* Dufour without considering either the insect itself or the species of willow.

3. This study has shown that several species of gall midges are responsible for this type of damage and that, so far as can be ascertained from an examination of cultivated species of willows, with *S. fragilis* in addition, each species of midge is restricted to one (in one case three) species of willow.

4. The adults, pupae and larvae of *R. saliciperda* Dufour, *R. triandraperda* sp.n., *R. purpureaperda* sp.n. and *R. justini* sp.n. have been described.

5. The bionomics of these species have been worked out. It has been found that, while all multiply by means of unisexual families, the first three species are single brooded but that *R. justini* sp.n. has two broods a year. *R. saliciperda* Dufour lives on *S. caerulea*, *S. fragilis* and *S. alba* (Cecconi), *R. triandraperda* sp.n. will only attack *S. triandra*, while *R. purpureaperda* sp.n. and *R. justini* sp.n. are restricted to *S. purpurea*.

6. The nature of the damage caused by the larvae of these midges has been described and control measures have been discussed. Tarring the stubs has been mentioned. It is suggested that cutting down the new growth in May, where practicable, would reduce the midge infestation. This latter treatment has the additional advantage of getting rid of initial caterpillar and frost damage which result in dead terminals and so produce side-branching close to the stubs. Wild Crack willow (*S. fragilis*) should be destroyed as it can act as a reservoir for *R. saliciperda* Dufour.

7. Keys have been drawn up for the identification of the midges using host plants, larval, pupal and adult female characters.

8. The following parasites are recorded—Torymidae: *Torymu* ssp., near *auratus* Fonsc.; Eurytomidae: *Eurytoma aciculata* Ratz., *E. saliciperdae* Mayr.; Pteromalidae: *Tridymus salicis* Nees; Eulophidae: *Pleurotropis*? *caenus* Walk., *Tetrastichus flavovarius* Nees, *T. roesellae* De Geer; Platygasteridae: *Platygaster cecidomyiae* Ratz., *P. sp.* (? *philinna* Walk.).



Fig. 1.



Fig. 2.



Fig. 3.



Fig. 4.

BARNES.—ON THE GALL MIDGES INJURIOUS TO THE CULTIVATION OF WILLOWS (pp. 86-105).



Fig. 1.



Fig. 2.



Fig. 3.



Fig. 4.

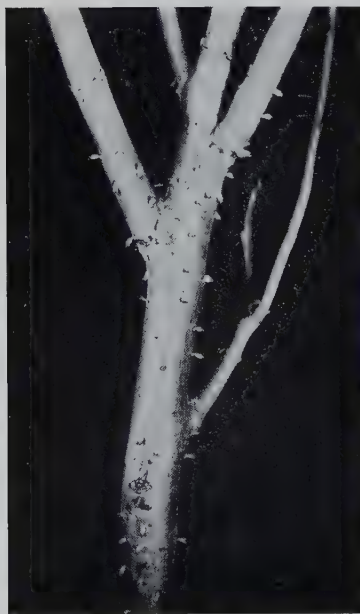


Fig. 5.



Fig. 1.



Fig. 2.



Fig. 3.



Fig. 4.



Fig. 5.

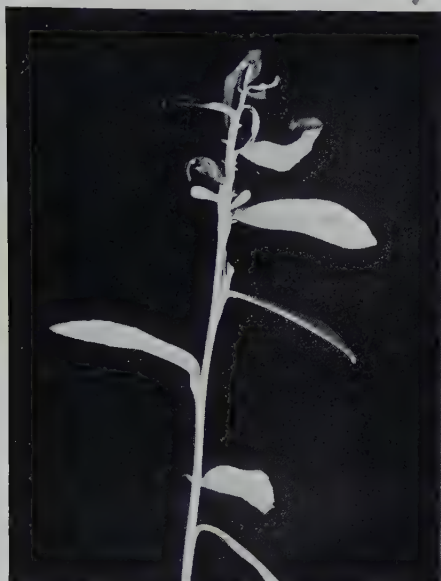


Fig. 1.



Fig. 2.

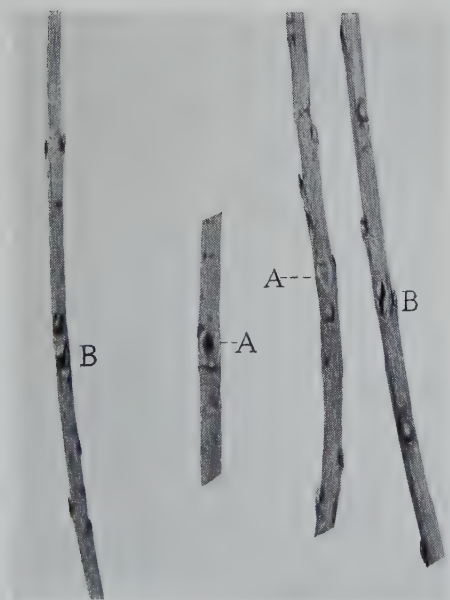


Fig. 3.



Fig. 4.

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EXPLANATION OF PLATES XI-XIV.

PLATE XI.

- Fig. 1. Adult ♀ *R. saliciperda* Dufour (Cecid. 2291, balsam mount). × 10·4.
 Fig. 2. Adult ♀ *R. triandraperda* sp.n. (Cecid. 1468, Berlese mount). × 10·4.
 Fig. 3. Adult ♀ *R. purpureaperda* sp.n. (Cecid. 2243, balsam mount). × 10·4.
 Fig. 4. Adult ♀ *R. justini* sp.n. (Cecid. 2272, balsam mount). × 10·4.

PLATE XII.

- Fig. 1. Damage to set of *S. caerulea* by larvae of *R. saliciperda* Dufour. Reduced.
 Fig. 2. Damage to branch of *S. caerulea* by larvae of *R. saliciperda* Dufour. Reduced.
 Fig. 3. Damage to branch of *S. caerulea* by larvae of *R. saliciperda* Dufour, cut to show larval chambers. Nat. size.
 Fig. 4. Damage to branch of *S. fragilis* by larvae of *R. saliciperda* Dufour. Reduced.
 Fig. 5. Empty pupal cases of *R. purpureaperda* sp.n. on *S. purpurea*. Nat. size.

PLATE XIII.

- Fig. 1. Eggs of *R. triandraperda* sp.n. × 134.
 Fig. 2. Newly hatched larva of *R. triandraperda* sp.n. × 134.
 Fig. 3. Damage to stub of *S. triandra* by larvae of *R. triandraperda* sp.n. Reduced.
 Fig. 4. Damage to stub of *S. triandra* by larvae of *R. triandraperda* sp.n., peeled to show larval chambers. Reduced.
 Fig. 5. Damage to sets of *S. triandra* by larvae of *R. triandraperda* sp.n. Reduced.

PLATE XIV.

- Fig. 1. Damage to *S. purpurea* by larvae of *R. justini* sp.n. Killed terminal shoot showing empty pupal cases. Nat. size.
 Fig. 2. Twig of *S. purpurea* illustrating *R. justini* sp.n., showing: A, pre-emergence window in stem; B, empty pupal case protruding from midvein on leaf. Nat. size.
 Fig. 3. Unpeeled rods of *S. purpurea* showing larval chambers of *R. justini* sp.n. Nat. size. A, discoloration indicating the presence of a larval chamber; B, larval chambers from which the larvae have been extracted by birds. Nat. size.
 Fig. 4. Peeled rods of *S. purpurea* showing larval chambers of *R. justini* sp.n. Nat. size.

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STUDIES ON APHIDES INFESTING THE POTATO CROP

III. EFFECT OF VARIATION IN RELATIVE HUMIDITY ON THE FLIGHT OF *MYZUS PERSICAE* SULZ.

BY W. MALDWYN DAVIES, B.Sc., PH.D.

(*University College of North Wales, Bangor.*)

(With 5 Text-figures.)

INTRODUCTION.

CONTRASTED differences shown to exist in the intensity and nature of the infestation of aphides attacking the potato crop in certain centres in North Wales^(1, 2, 3) prompted a detailed study of the factors involved. Each year, since 1928, the total population of aphides on the potato crop has been much greater in the eastern portion of the province, viz. certain centres in Flintshire, than in the westerly districts of south Caernarvonshire and the island conditions of Anglesey. Details of the infestations have been published in Part II of this series⁽³⁾ so that for the purpose of this present paper it will suffice to state that while at these Flintshire centres the population at a given date has ranged from 500 to 1300 *Myzus persicae* per 100 leaves taken at random, that of south Caernarvonshire has never exceeded 90 and more commonly has been below 40 per 100 leaves. While these infestations consisted largely of apterae, the counts were taken at a time when they were indicative of the number of alatae which initially arrived on the potato crop at the respective centres. In 1933, however, preliminary experiments were conducted in the trapping of migrating winged aphides. Migrating alatae were trapped on adhesive netting throughout the season at centres in each district. The results, which are being confirmed in detail and will be published separately, show very clearly that the total numbers of migrating alatae of a wide range of species of aphides were much greater per unit area in the eastern than in the western districts. From the previous counts of apterae this might have been expected but the enormous contrast, even in a dry season such as 1933 when in the western districts aphides were more common than usual, could hardly have been anticipated. During the period of maximum migration, viz. June and July, the number of

alate aphides taken on the traps in Flintshire was forty times greater than the number taken at south Caernarvon. It was thus evident that some limiting factor or factors were involved inhibiting the migration of aphides in the case of the south Caernarvonshire district. Had the contrast in numbers been confined to *M. persicae* an explanation would have been forthcoming in the comparative scarcity of large areas of the common winter host plants, winter brassicae. Flintshire is nearer large market-garden districts with acres of winter brassicae known to harbour hibernating *M. persicae* in quantity, whereas in south Caernarvon wintering brassicae are confined to private gardens. But the traps revealed the fact that migrating alatae of such species as *M. crataegarium* Walk., *Anuraphis padi* (L.), *Cavariella aegopodii* (Scop.), the winter hosts of which abound in south Caernarvonshire, were equally scarce in that district as compared with the eastern localities. This evidence, confirming as it does field observations under the varied conditions during 1928-33, points clearly to some factor operating against the migration of winged aphides in the south Caernarvonshire and allied districts which is absent from the Flintshire areas.

RELATIVE HUMIDITY OF SOUTH CAERNARVONSHIRE AND FLINTSHIRE.

A knowledge of the two particular districts suggested that so far as meteorological factors were concerned most contrast would be found in the degree of humidity in the different localities. Light, temperature and, to a lesser extent, wind—factors observed to have an influence upon the flight of alatae—are generally similar in these two districts during the period of migration. There is, however, clear evidence of the high humidity of south Caernarvonshire, for the growth of lichens and mosses on all trees and shrubs is excessive and characteristic of the district. This condition, which is indicative of a moist atmosphere, is not observed in Flintshire. More particularly, however, the south Caernarvon area is very subject to heavy sea mists from the Atlantic, such condition being rarely met with in Flintshire. Through the kindness of the Meteorological Officers at Pwllheli, south Caernarvonshire, and the R.A.F. Station, Sealand, Flintshire, it has been possible to compare the Mean Monthly Values for Relative Humidity, taken at 9 a.m., during the months May to August for the years 1928-33. The records, shown graphically in Fig. 1, show clearly that the relative humidity in south Caernarvonshire is consistently and appreciably above that of Flintshire at the time when migration of aphides is taking place.

EXPERIMENTS ON THE FLIGHT OF *MYZUS PERSICAE*
AT VARYING RELATIVE HUMIDITIES.

While the correlation between the several meteorological factors and flight of aphides *in the field* is being further studied, it was necessary to ascertain the precise effect of each meteorological factor under the controlled conditions of the laboratory. It was decided in the first place to ascertain the effect of relative humidity upon the flight of *M. persicae*.

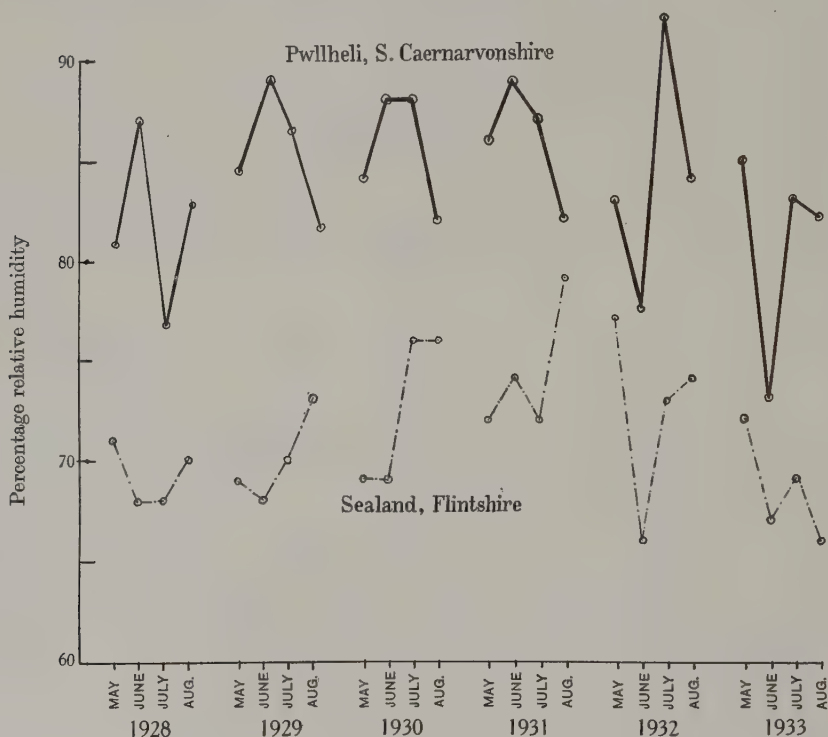


Fig. 1. Mean monthly value of relative humidity.

TECHNIQUE.

Large numbers of alate *M. persicae* were bred on sprouting potatoes on trays covered with muslin. The ability of the winged specimens to fly was ensured by allowing the aphides to fly from the trays to a closed window. They were then collected, as required, in batches of 25 and placed in glass tubes ready for immediate use.

Preliminary experiments showed that appreciable changes in the intensity of light affected flight, so that a source of light of constant intensity was essential for these experiments. This was obtained by using a 500 watt lamp and controlling the current by means of a rheostat and an ammeter. The current was kept constant at 1.9 amps, giving a light of 360 Mean Spherical Candles. The experimental chambers were placed 18 in. below the suspended light. The temperature was maintained constant in an enclosed dark room by means of a range of radiators and the room temperature was checked by a series of thermometers. The experiments were conducted at a series of temperatures, viz. 55° F., 70° F., 80° F. and 90° F. Variation in relative humidity of the air was secured by the use of a series of solutions of sulphuric acid with a selected range of specific gravities (4, 5). The series consisted of 0, 25, 50, 70, 85, and 100 per cent. relative humidity. These humidities were obtained in a series of desiccators used as experimental chambers. Each desiccator had a sheet of perforated white paper covering the usual wire-gauze, so preventing the aphides falling into the sulphuric acid below. The usual lid of the desiccator was replaced by a sheet of thin clear plate-glass (22 plate), in the centre of which was bored a hole 1 inch in diameter. This glass sheet was efficiently sealed to the desiccator by means of plasticine. The hole in the centre contained a cork through which was inserted a thermometer so that the temperature inside the chamber could be accurately ascertained during the experiments. The hole also served as the entrance for the winged aphides. After collection, the twenty-five aphides in the glass tube were allowed to remain at the temperature of the experimental room for half an hour before being inserted rapidly into the experimental chamber. They were again allowed seven minutes in the experimental chamber to become accustomed to the particular humidity before records of flight were taken. The recording of flights involved observing in the respective experimental chambers the number of flights (*i.e.* instances when aphides took to the wing) made by each batch of twenty-five aphides during the unit period of one minute. Each observation was repeated, consecutively, twelve times, giving one series of observations of twelve-minute duration. In order to obtain a close comparison the observations in two humidities were carried out at the same time by the writer and his assistant, each recording counts in the respective chambers. Any personal error was guarded against by exchanging the chambers examined at the sixth minute. In practice, however, the flights occurred in such a manner that it was possible to record with ease each instance of flight.

RESULTS.

The results of the experiments are expressed graphically in Figs. 2-5.

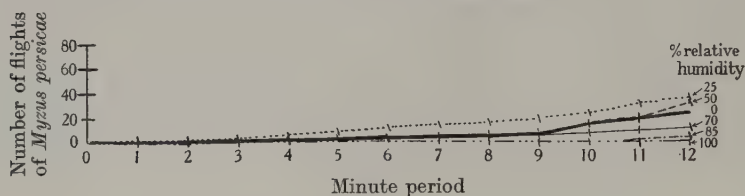


Fig. 2. Rate of flight of *Myzus persicae* at 55° F.

(a) EFFECT OF VARYING HUMIDITY UPON FLIGHT
AT A TEMPERATURE OF 55° F.

At a temperature of 55° F. (Fig. 2), under the conditions of the experiment, winged *M. persicae* were sluggish and reluctant to take to the wing. Variation in relative humidity, therefore, did not effect flight when the temperature was the limiting factor. In the lower humidity chambers (0, 25 and 50 R.H.) flight was slightly in excess of that in the higher humidities, but in no case did it average more than three flights per minute.

(b) EFFECT OF VARYING HUMIDITY UPON FLIGHT
AT A TEMPERATURE OF 70° F.

It was evident from the behaviour of the aphides at 70° F. (Fig. 3) that this temperature was favourable for flight, so that the effect of variation in relative humidity became apparent. In the lower humidities flight was frequent, averaging as follows: 0 per cent. R.H., 25.0 flights per minute; 25 per cent. R.H., 32.3 f.p.m.; 50 per cent. R.H., 20.7 f.p.m. At 70 per cent. R.H. it was evident that humidity definitely reduced the number of flights which only averaged 13.5 f.p.m. This retarding effect was more striking as the humidities increased, for at 85 per cent. R.H. flights averaged 6.2 f.p.m. and at 100 per cent. R.H. flights had ceased, for the aphides remained more or less stationary on the paper or sides of the chamber and there was no attempt made even to extend their wings.

(c) EFFECT OF VARYING HUMIDITY UPON FLIGHT
AT A TEMPERATURE OF 80° F.

With the increase in temperature the effect of relative humidity upon flight became marked. The favourable temperature induced considerable flights in the lower humidities attaining as high a figure as 55 flights per minute at 0 per cent. R.H. The extent of the flights is seen in Fig. 4 when

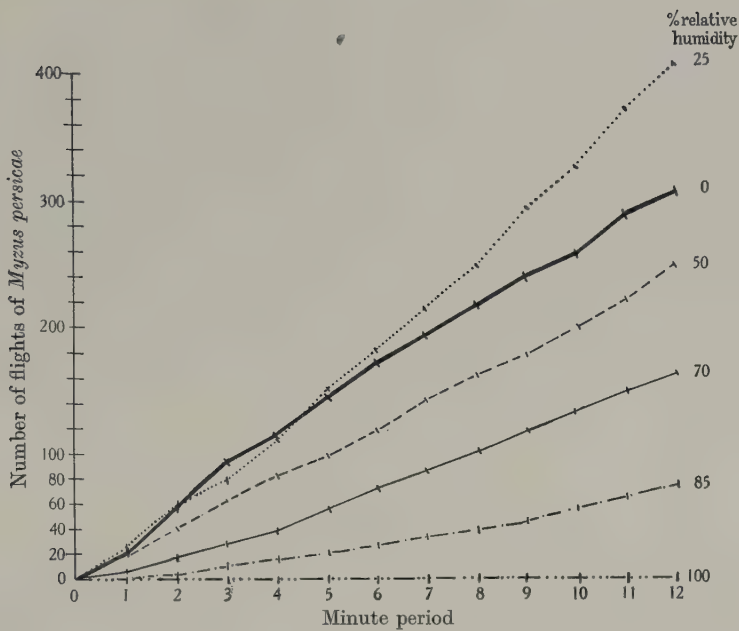


Fig. 3. Rate of flights of *Myzus persicae* at 70°F.

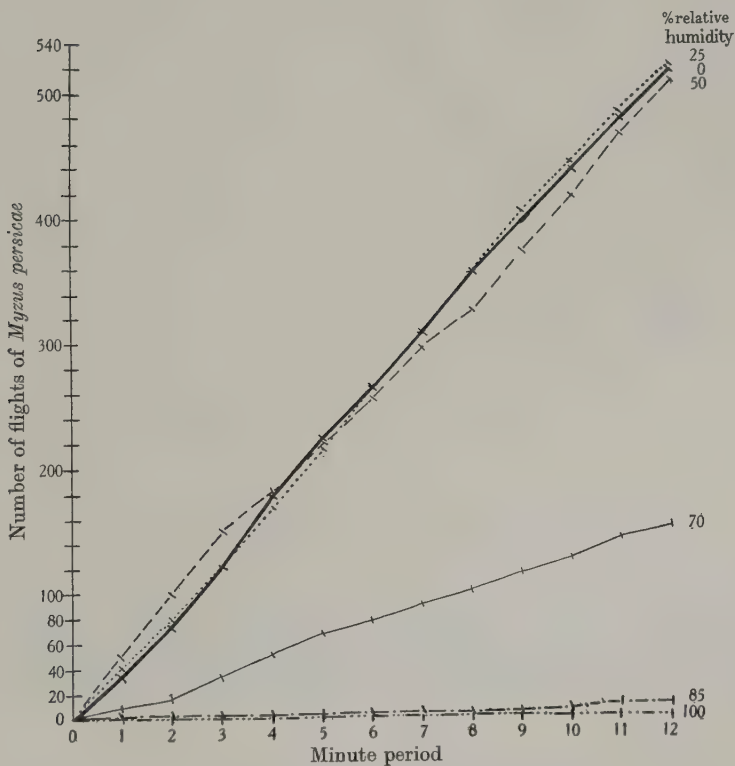


Fig. 4. Rate of flight of *Myzus persicae* at 80°F.

the averages were as follows: 0 per cent. R.H., 42.0 f.p.m.; 25 per cent. R.H., 43.8 f.p.m.; 50 per cent. R.H., 42.5 f.p.m. At this temperature there was a very marked contrast in the number of flights in the higher humidities compared with that in the chambers of low humidity. Such effect is clearly demonstrated in the graph (Fig. 4). The average flights in the higher humidities were: 70 per cent. R.H., 12 f.p.m.; 85 per cent. R.H., 0.5 f.p.m. and in 100 per cent. R.H. there was again no indication of flight.

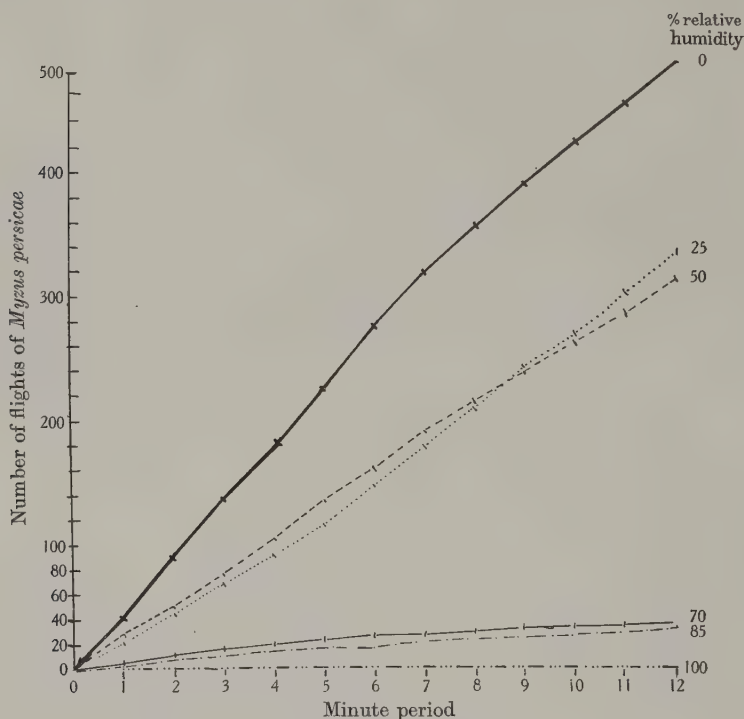


Fig. 5. Rate of flight of *Myzus persicae* at 90°F.

(d) EFFECT OF VARYING HUMIDITY UPON FLIGHT
AT A TEMPERATURE OF 90°F.

It was evident that the temperature of 90° F. induced great activity, for in all chambers the aphides crawled about rapidly. The aphides readily took to the wing in the lower humidities and considerable as the number of flights was it would have been even greater had it not been

that the aphides were prone to crawl actively (run is a better description of the action) when not in flight. The average number of flights in the lower humidities was as follows: 0 per cent. R.H., 40.8 f.p.m.; 25 per cent. R.H., 28.2 f.p.m.; 50 per cent. R.H., 26.6 f.p.m. At this higher temperature the inhibiting effect of humidity upon flight was even more striking, for in 70 per cent. R.H. the average number of flights per minute had fallen to 3.3. In the 85 per cent. R.H., flights were more in the nature of falls rather than flights and even then only averaged 3.1 f.p.m. At 100 per cent., although the aphides continued to crawl actively, the total flights were confined to one solitary flight in the first minute.

DISCUSSION.

While the precise figures for flight in this particular type of experiment are conditional upon the set of conditions pertaining to the experiment, the results demonstrate clearly that, when temperature and light are favourable (and these factors are normally favourable during the months of June and July), the humidity of the atmosphere has a very marked effect upon the flight of *M. persicae*. It is evident that above a temperature of 55° F.—approximately a minimum temperature during the daytime of June and July—a relative humidity of 70 per cent. and above will markedly reduce the instances of flight by *M. persicae*. At higher temperatures of 80° F. and 90° F., the effect of humidity is even more marked and flight is negligible when the humidity exceeds 85 per cent. The survey of the aphid population on the potato crop conducted in North Wales since 1928 has shown (1-3) that high altitudes, with bleak exposed conditions, are not necessarily the conditions in which aphides are scarce. Such an explanation has been assumed in the case of those areas in Scotland which are noted for their high quality seed potatoes. Centres of high altitudes are known in North Wales where aphides are common on potatoes and increase in virus diseases has been appreciable. On the contrary, the districts in which low infestations have been consistently recorded are low-lying, often almost at sea-level. In some instances the centres have been exposed but this has been by no means general. Where data has been available for the centres it has been seen that high relative humidities are prevalent and, from the evidence of the present experiments, would account, through the inhibition of migration of alatae, for the low initial infestation of aphides on the potatoes. It is

significant that at the centres in south Caernarvon the population of *M. persicae* in 1933⁽³⁾ was over twice as high as in any of the previous five years. It attained the figure of 90 *M. persicae* per 100 leaves. From Fig. 1 it will be seen that this high infestation can be correlated with the low mean value for relative humidity which in June 1933 was considerably lower than at any time in the previous five years. Further, this influence of humidity upon flight is, no doubt, the explanation of the fact that in North Wales infestations of aphides are always greater following a period of east winds. From an examination of the meteorological data at the College Farm it is seen that the humidity is definitely lower on days when east winds are blowing compared with the humidity during the prevailing south-west winds.

SUMMARY.

1. Contrasted differences in the intensity and nature of the infestation of aphides attacking the potato crop in certain districts in North Wales prompted a detailed study of the factors involved.

2. Migrating alatae are considerably more numerous in the Flintshire districts than in the south Caernarvon area. The mean monthly values for relative humidity are consistently and appreciably lower in the former district than in the latter.

3. Controlled laboratory experiments on the effect of variation in relative humidity upon the flight of *M. persicae* clearly demonstrate an inhibitive action of high humidities upon flight. The experiments were conducted at 0, 25, 50, 70, 85 and 100 per cent. relative humidities at the temperatures 55° F., 70° F., 80° F. and 90° F.

4. The findings of the survey of aphid population at centres in North Wales during 1928-33 are discussed in the light of these results.

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BIOLOGICAL STUDIES OF CERTAIN SPECIES OF *CALIROA* COSTA AND *ENDELOMYIA* ASHMEAD (HYMENOPTERA SYMPHYTA)

BY HERBERT W. MILES, M.Sc., Ph.D.

(Victoria University of Manchester.)

(With Plates XV and XVI and 3 Text-figures.)

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INTRODUCTION.

SAWFLIES of the genus *Caliroa* are widely distributed throughout the world and are of considerable economic importance. Larvae of *C. limacina* and *C. annulipes* are leaf skeletonisers; they destroy one epidermis and the mesophyll, usually leaving the other epidermis intact. The adults frequent the undersides of the leaves of their host plants and, when disturbed, fold their legs, wings and antennae and drop to the ground. Larvae of *C. limacina*, commonly known as the pear and cherry slug-worms, infest pear, plum, cherry, hawthorn, quince and allied trees. The insect occurs in Europe, North America, South Africa, Australia and New Zealand, and in some of these regions is a pest of major importance. *C. annulipes* occurs in both Europe and North America, and the larvae commonly infest *Salix* sp.; it is also recorded from lime, oak, birch and hazel, and the writer has taken larvae feeding on cherry and hawthorn. *Endelomyia aethiops* infests *Rosa* sp. in Europe and North America. Larvae of this species are also leaf skeletonisers and during 1933 and 1934 have been locally serious as a pest of cultivated roses.

CALIROA LIMACINA RETZ.¹ (PEAR AND CHERRY SAWFLY).

C. limacina Retz. was studied in some detail by Peck in America as early as 1799, and much of the information he gave was verified by Marlatt⁽¹⁵⁾ who published an account of the life history in 1897. Dyar⁽⁷⁾ reared larvae and published data on the number of moults and the size of larval heads; and Loisele⁽¹²⁾ in France noted that in his breeding experiments he could only obtain females. Parthenogenesis in *C. limacina* was studied by Dobrodiev⁽⁸⁾ in Russia and Ewing⁽¹⁰⁾ in America. In England there has been no detailed study of the species, but contributions to our knowledge have been made by Theobald⁽²³⁾, Chapman⁽⁵⁾, and Adkin⁽¹⁾.

Material for the present study has been obtained mainly in Lancashire and Cheshire, and acknowledgments are due to Mr W. B. Mercer, B.Sc., for special facilities for collecting and observing the species at the Cheshire School of Agriculture.

Description. Adults measure 5-7 mm. from the front of the head to the tip of the abdomen. Head, thorax and abdomen are shining black, and the head and thorax are sparsely and shallowly punctured. The pentagonal area is fairly well defined and passes without a transverse frontal ridge to the supra-antennal region. The posterior legs are black; the tips of the femora, tibiae and tarsi of the anterior legs may be brownish; and the tarsi of the intermediate legs may be obscurely dark brownish. The forewings of the female are infuscated throughout, conspicuously across the wing behind the stigma and faintly towards the base and apex. The stigma and veins are dark brown to black. The radial cross vein² is interstitial with R 4, or meets R 3 immediately before its junction with R 4. The medio-cubital cross vein bends sharply at about two-thirds of its length from the medius and meets the cubitus almost at right angles. Cu 1 leaves the stem at a point about one-third of the distance along the base of cell M 4. The hindwings usually have two enclosed median cells, R + R 4 + 5 and M 4 + 1st M 2, though the latter is not always completely enclosed owing to the disappearance of part of M 2 at its junction with R 4 + 5 + M 1. The forewing of the male resembles that of the female, but in the hindwing some of the transverse veins are suppressed.

Flight period. From examination of data attached to available specimens, adults appear to be on the wing from about the middle of June and are most commonly taken in June and July. Ormerod⁽¹⁷⁾ records

¹ This insect was formerly known as *Eriocampa* or *Eriocampoides limacina*.

² The nomenclature of the veins is as given by MacGillivray⁽¹⁴⁾.

infestation by *C. limacina* larva as early as June 14th and actually received larvae on June 19th, 1893, but these dates seem to indicate an unusually early appearance of the adults. At the end of July and the beginning of August there is a break in the records and this seems to indicate a period between the emergence of adults from overwintered larvae and the emergence of adults of the second generation from larvae that have reached maturity during the earlier part of the season. The following records show that *C. limacina* is on the wing for a considerable period:

June 10th, 1934	Nantwich, Ches.	July 24th, 1930	Stockport
" 16th, 1923	Skirwith, Cumb.	" 24th, 1873	Worcester
" 17th, 1932	Preston, Lancs.	" 25th, 1929	Chester
" 26th, 1932	Preston, Lancs.	Aug. 9th, 1934	Nantwich, Ches.
" 28th, 1932	Skirwith, Cumb.	" 15th, 1933	Chester
July 9th, 1932	Cornwall	" 17th, 1932	Birmingham
" 10th, 1934	Chester	" 24th, 1933	Nantwich, Ches.
" 18th, 1930	Chester		

While the date of appearance is not likely to vary much throughout England, there is some evidence that the time of emergence is influenced by climatic conditions. Marlatt⁽¹⁵⁾ in America records adults as early as mid-April at Washington, D.C., lat. 39° N., but not until a month later at Boston, Mass., lat. 42° N. England lies north of the 50th parallel of latitude and emergence does not generally begin until June.

In Lancashire and Cheshire during 1928-34 adults of *C. limacina* were first observed in June and continued on the wing until the latter part of August. During July and August, adults, eggs and larvae in various stages of development occurred on the host plants. Adults taken up to the latter part of July probably emerged from overwintered larvae, but adults taken in August are likely to have emerged from larvae that reached maturity earlier in the same season.

Oviposition. In bright sunshine the adults are very restless and readily take flight, but in dull weather they drop from the foliage when disturbed. During the period of oviposition they run actively over the foliage of the host plant, their short vibrating antennae continually testing the surface. At egg-laying an incision is made in the lower epidermis of the leaf and the ovipositor gradually exerted and thrust obliquely forward into the leaf tissue until the insect leans far to one side in a characteristic position. Observations on the path of the ovipositor show that it is first directed towards the upper surface of the leaf and then a more or less circular incision is made between the mesophyll and the upper epidermis. After a short pause the egg is passed into the prepared cavity. The rather flattened oval or circular outline of the egg is discernible on the upper leaf surface, but only the incision can be seen on

the under surface. In some cases the ovipositor ruptures the upper epidermis and as a result the egg may be wholly or partly protruded on to the upper surface of the leaf, as shown in Plate XV, fig. 2. When exposed on the leaf surface the eggs are oval and yellowish.

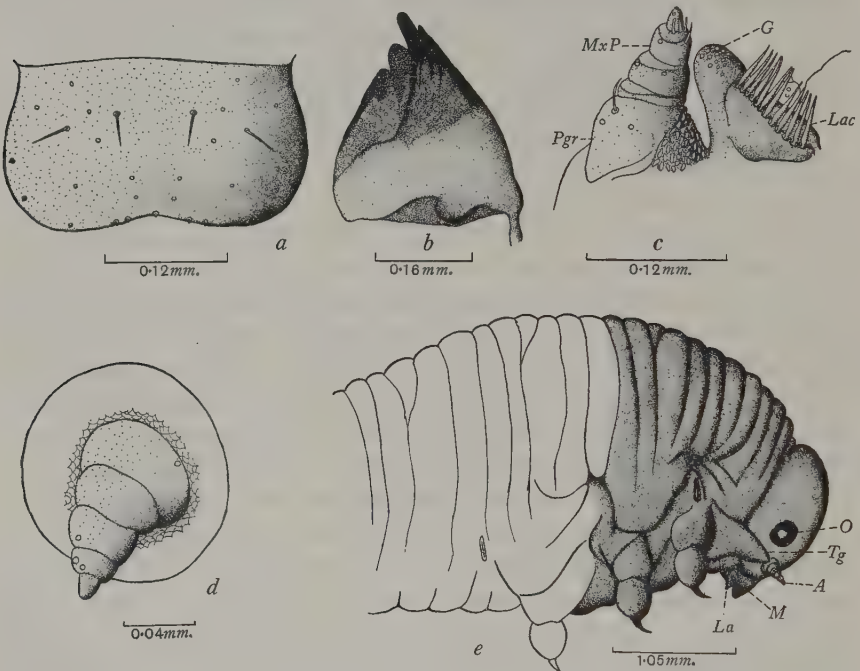
The insects oviposit readily on both pear and cherry, usually selecting the lower leaves on extension shoots of the current season's growth. Insects bred from larvae reared on cherry were placed in breeding cages in which shoots of pear and cherry in approximately similar stages of development were available. It was found that 198 eggs were laid in pear leaves and only 68 eggs in the leaves of cherry. This suggests that pear may be the more favoured host plant, but the predilection is probably not great and the availability of either host when the adults are active is sufficient to stimulate oviposition. Insects in captivity lived 6-8 days and laid 40-50 eggs.

Incubation period. In the laboratory the incubation period was 9-11 days. Eggs laid on June 13th hatched on June 22nd; those laid on June 26th hatched on July 6th, and those laid on July 26th hatched on August 6th. In the fruit plantation eggs laid on June 10th-13th, 1934, hatched on June 24th after an incubation period of 11-14 days. During the incubation period the upper epidermis lying over the egg gradually dries and becomes slightly discoloured, thus making the egg more conspicuous. The swelling of the egg during incubation, a phenomenon observed in *Pristiphora pallipes* Lep.⁽¹⁶⁾ and other sawflies, was only slight.

Description of larvae (Text-fig. 1). The larvae emerge on the upper surface of the leaves and begin feeding almost immediately on the upper leaf tissue. At eclosion they are a dull yellowish colour but gradually they become covered with dark slime. At first the slime only partly covers the dorsal surface, but in time it extends over the back and sides and becomes darker and thicker. The dark slimy covering, the small retracted head, the large thorax and tapering body cause the insects to resemble small black slugs. At ecdysis the slime is shed with the exuvia. Immediately after ecdysis the larvae are dull yellowish, but the characteristic coating of slime is soon secreted and the form and colour of the larvae obscured. During the first instar the larval head is pale yellowish but subsequently it becomes dark brown.

Larvae leaving the host plant for pupation sites have no coating of slime. They measure 10-12 mm. and are yellowish, with the head paler than the body and the tips of the mandibles black or dark brown. The antennae have five segments and are rather prominent. The labrum is emarginate and the mandibles long and bluntly toothed. The maxillary

palpi have four segments, the galeae are short and blunt, and the laciniae narrow and flattened and fringed with setae. The labium is fleshy and the spinneret protrudes on its upper surface. The labial palpi have three segments and occur at the sides of the labium just below the spinneret. The spiracles are long and narrow and placed low on the sides of segments 1 and 4-11 inclusive. Prolegs occur on segments 5-11, but are absent from the last segment and the tip of the body is slightly elevated. The thoracic



Text-fig. 1. Details of larva of *Caliroa limacina*. *a*, dorsal view of labrum. *b*, right mandible. *c*, left maxilla; *MxP*=maxillary palp; *Pgr*=palpiger; *G*=galea; *Lac*=lacinia. *d*, antenna. *e*, head and thorax; *O*=ocellus; *A*=antenna; *M*=mandible; *La*=labium; *Tg*=prothoracic gland.

legs are short and stout with dark slender simple tarsal claws. A pair of conical glands arise near the bases of the prothoracic legs. The glands are flattened on their inner surface and project forwards, and when the head is bent in the normal position for feeding they curve towards the mouth. Chapman (5) describes these glands and associates them with feeding. In *C. limacina* the annulets tend to be narrow and unbroken, and in segments 4-11, where the annulation appears to be constant, each segment has six annulets.

Larval development. Larvae of the first generation of *C. limacina* develop rapidly. In the feeding stage they have six ecdyses. No feeding takes place after the sixth ecdysis, but shortly after sloughing the skin and coating of slime the larvae enter the soil to construct cocoons. The following data show that larvae reach maturity in 18–21 days after eclosion.

Table I.

Larva No.	Date of hatching	Duration of larval stages (in days)						Total days
		1st	2nd	3rd	4th	5th	6th	
1	June 23	5	4	2	2	3	4	20
2	"	5	4	3	2	3	3	20
3	"	5	4	2	2	3	4	20
4	"	5	4	2	2	3	5	21
5	"	5	4	3	2	2	3	19
6	June 24	5	3	3	2	3	2	18
7	"	5	3	2	3	2	4	19
8	"	5	3	3	2	3	3	19
9	"	5	3	3	3	2	3	19
10	"	7	2	2	3	2	3	19

In the larvae under observation growth was fairly regular. The species is thelytokous, and since all the larvae were parthenogenetically produced, measurements were not complicated by variation in the number of instars. The following is a summary of the data on growth, and shows an average growth ratio of 1.25.

Instar	Average head width in mm.	Growth ratio	Average frons width in mm.	Growth ratio
1st	0.40	—	0.15	—
2nd	0.56	1.40	0.19	1.26
3rd	0.74	1.32	0.23	1.21
4th	0.94	1.27	0.30	1.30
5th	1.06	1.13	0.35	1.16
6th	1.23	1.16	0.43	1.23

Pupation. Fully fed larvae enter the soil to make cocoons soon after the sixth ecdysis. By this time they have started to contract and appear deeply wrinkled, yellowish orange in colour and quite free from the slimy coating characteristic of the earlier instars. The cocoons are oval and measure rather less than a quarter of an inch in length. They are usually constructed at a depth of about 2 in. and are only rarely deeper than 3 in. in ordinary garden or fruit plantation soil. Wilson⁽²⁴⁾ has already pointed out that the cocoons consist mainly of soil particles with only sufficient saliva to make them adhere; thus they have little of the tough parchment-like substance commonly found in cocoons constructed in the soil by Nematine sawflies and are so fragile that they are frequently broken during cultural operations. Within the cocoon the larva contracts

until it measures only about 5 mm. Table II shows that under some conditions larvae of the first generation pupate almost at once and produce a second generation of adults in 4-5 weeks, but others remain in the prepupal condition until the following year. The pupal stage lasts 2-3 weeks.

Table II.

Date of cocooning	Date of emergence	Period in days	Pupal stage in days
2. viii. 28	24. vii. 29	356	—
29. vii. 29	18. vii. 30	354	—
27. vii. 33	10. vi. 34	318	20
27. vii. 33	10. vi. 34	318	20
27. vii. 33	12. vi. 34	320	18
28. vii. 33	14. vi. 34	321	14
13. vii. 34	15. viii. 34	33	—
13. vii. 34	15. viii. 34	33	—
13. vii. 34	15. viii. 34	33	—
14. vii. 34	15. viii. 34	32	—
14. vii. 34	9. viii. 34	26	—
14. vii. 34	9. viii. 34	26	—

Of 30 larvae that matured in July 1934, 11 emerged as adults during the following month and the rest continued in the cocoons in the prepupal stage throughout the summer and then hibernated. Although Theobald⁽²³⁾ records two generations of *C. limacina* as the normal condition, observations over a period of years indicate that the species is not always bivoltine in the north of England. The season of 1934 was characterised by high temperatures during June and early July and this may have speeded up the emergence of the first generation of adults and stimulated larval development early in the season. Attention has already been drawn by Marlatt⁽¹⁵⁾ to the fact that all individuals of the first generation do not emerge the same season and the present observations verify this.

Parthenogenesis. *C. limacina* appears to rely for reproduction on parthenogenetic thelytoky, and in the course of breeding experiments and field collecting since 1928 a male has occurred only once.

Economic importance. In England larvae of *C. limacina* occasionally occur in serious numbers on the foliage of pear and cherry in commercial fruit plantations, and spraying is then necessary to control them. More usually, however, the insects are found in numbers in fruit tree nursery beds where rapidly growing host plants seem to offer specially favourable conditions, and in gardens where spraying is not carried out as a routine measure. Plums are occasionally attacked but pear and cherry seem to be the favoured host plants and Morello cherries are generally more severely attacked than other varieties.

In 1929 in Cheshire a number of pear trees of different varieties growing together in mixed rows were infested with pear sawfly. It was

observed that the intensity of attack varied considerably in the different varieties, "Conference" being badly infested; "Dr Jules Guyot," "Fertility" and "Marie Louise d'Uccle" infested to a much less extent, and the variety "Clap's Favourite" appeared almost immune. Whether this was owing to selection on the part of the sawflies was not determined, but it seems possible that the toughness of the epidermis of the leaves might be an important factor when a choice of sites for oviposition is available.

CALIROA ANNULIPES KLUG.

Description. *C. annulipes* Klug. and *C. limacina* Retz. are easily distinguished, since the former species always has some white on the posterior legs. *C. annulipes* differs from *C. varipes* Klug. in having the junctions of the radial cross vein and R4 with R3 widely separate in the forewings.

Adults of *C. annulipes* are 5-7 mm. long and have a wing expanse of 9-11 mm. They are black except for the bases of the tibiae and tarsi which may be whitish or light brownish. In the female all the tibiae are distinctly and often extensively white at the base and the tarsal segments are also whitish. The white colour is most evident in the posterior legs where it extends over more than the basal third of the tibiae and the basal half of the metatarsal segment. In the male the tarsi of the anterior and intermediate legs may be extensively whitish or light brownish, but the tarsi of the posterior legs are entirely black and the light coloration on the tibiae is reduced to a narrow basal band or may almost disappear.

In both sexes the forewings are infuscated on the basal two-thirds, the infuscation being most marked across the wing behind the stigma. The apices of the wings are almost hyaline. The cells R + R4 + 5 and M4 + 1st M2 are present in the hindwings of both sexes, and the vein M3 joins A1 at or before the junction of A1 and A2, in this respect closely resembling the hindwings of *C. limacina*.

Oviposition and incubation period. Adults of the first generation of *C. annulipes* are on the wing in late May and early June. This species differs from *C. limacina* in egg-laying habits. Females of *C. annulipes* take up a position on the upper surface of the leaf and, inserting the ovipositor through the upper epidermis and the mesophyll, deposit the egg in a cavity just above the lower epidermis. On the under side of the leaf the egg is visible as a slight circular swelling similar to that made by the egg of *C. limacina* on the upper side of the leaves of its host plants. Two or three eggs per leaf is the usual number and they are frequently placed

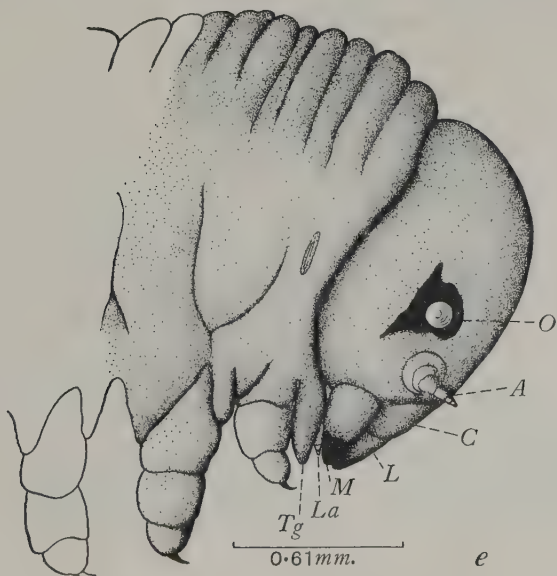
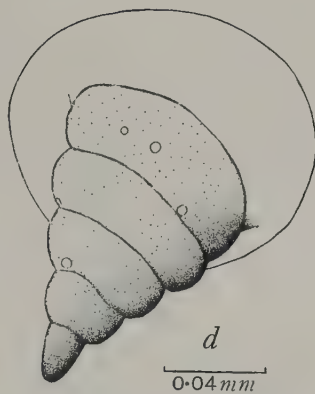
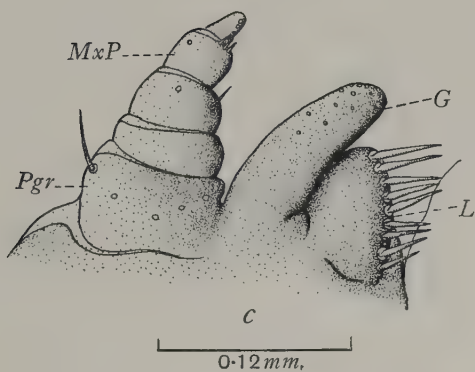
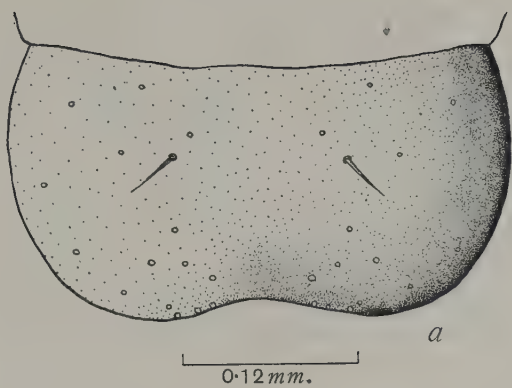
nearer the midrib than the leaf margin. After an incubation period of 13–15 days the larvae escape through a crescent-shaped opening on the lower surface of the leaf.

Description of larvae (Text-fig. 2). Unlike the larvae of *C. limacina* the larvae of *C. annulipes* are repelled by light and feed in the shade on the under sides of the leaves. They are at first translucent with the head dark and the thorax enlarged and the whole body covered with almost transparent slime. Older larvae become faintly yellowish or parchment coloured, with the greenish contents of the alimentary canal visible through the integument. The larvae develop fairly rapidly and reach maturity in 19–22 days. Growth was more complicated in *C. annulipes* than in *C. limacina* because in breeding experiments with *C. annulipes* both sexes were present, and larvae becoming males had one ecdysis less than those becoming females. The average growth ratio of the head capsules of successive instars was 1.24, which is practically identical with that of *C. limacina*. Larvae of *C. annulipes*, however, do not appear to moult on the cessation of feeding, but prior to entering the soil for cocooning the slimy coating dries up and the green contents of the alimentary canal are completely digested.

When ready to enter the soil the larvae measure 11–12 mm. and are dull whitish or parchment coloured, with the integument deeply wrinkled. The head is brown, darkly margined dorsally and laterally, and retracted into the thorax. The eyes are black, and the antennae have five segments and are rather prominent. The thorax is large and extends forwards around the head, and the abdomen tapers sharply. The thoracic legs are brownish, the first pair being rather smaller than the second and third pairs. The tarsal claws are simple, slender and curved. The prothoracic glands are more elongate than in *C. limacina*. They project forwards and inwards, often nearly across the retracted lower part of the head. Prolegs are present on segments 5–11 inclusive and there are no caudal prolegs, their position being occupied by a slight median swelling.

Pupation. After the cessation of feeding the larvae enter the soil for pupation. The cocoons are made of soil particles cemented with salivary secretion and are much stouter than those of *C. limacina*. Within the cocoon the larva contracts until it measures only about 5 mm. before pupation. For larvae of the first generation the period spent in the soil

Text-fig. 2. Details of larva of *Caliroa annulipes*. *a*, dorsal view of labrum. *b*, right mandible. *c*, left maxilla. *d*, antenna. *e*, head and part thorax; *C*=clypeus (other lettering as in Text-fig. 1*e*).



is 16–18 days but larvae of the second generation hibernate in the cocoons in the contracted prepupal condition.

Annual cycle. Loisele (11) made observations on the annual cycle of *C. annulipes* in France. He found larvae of the first generation early in July, and these matured about mid-July and gave rise to adults about the end of the month. He also recorded the second generation of larvae as mature by the end of September. Perkins (18) states that in Devon the species may have more than two broods in the year. In the north-west of England *C. annulipes* appears definitely bivoltine. Up to the present the writer has had no instances of larvae of the first generation hibernating and there have been no signs of a third generation in the season. During July and August both adults and larvae may be found, and larvae of the second generation continue feeding until late in September. Unlike *C. limacina*, males of *C. annulipes* occur frequently in both the first and second generations of adults, thus indicating that reproduction does not depend entirely upon parthenogenesis.

Host plants. Enslin (9) lists *Quercus*, *Tilia* and *Betula* as host plants of *C. annulipes*. Cameron (4) bred the species from *Salix viminalis* and the writer has taken the larvae on *S. viminalis*, *S. caprea*, *Prunus cerasus* and *Crataegus oxyacantha*. *S. viminalis* and *S. caprea*, however, appear to be the usual hosts. In 1933 and 1934 the larvae were found feeding on Morello cherry in Cheshire in association with the slugworm, *C. limacina*. Reh (20) records *C. annulipes* as causing injury to lime trees, but there appears to be no English record of this tree as a host plant. Perkins (18) lists oak and birch as host plants in Devonshire.

ENDELOMYIA AETHIOPS FAB.

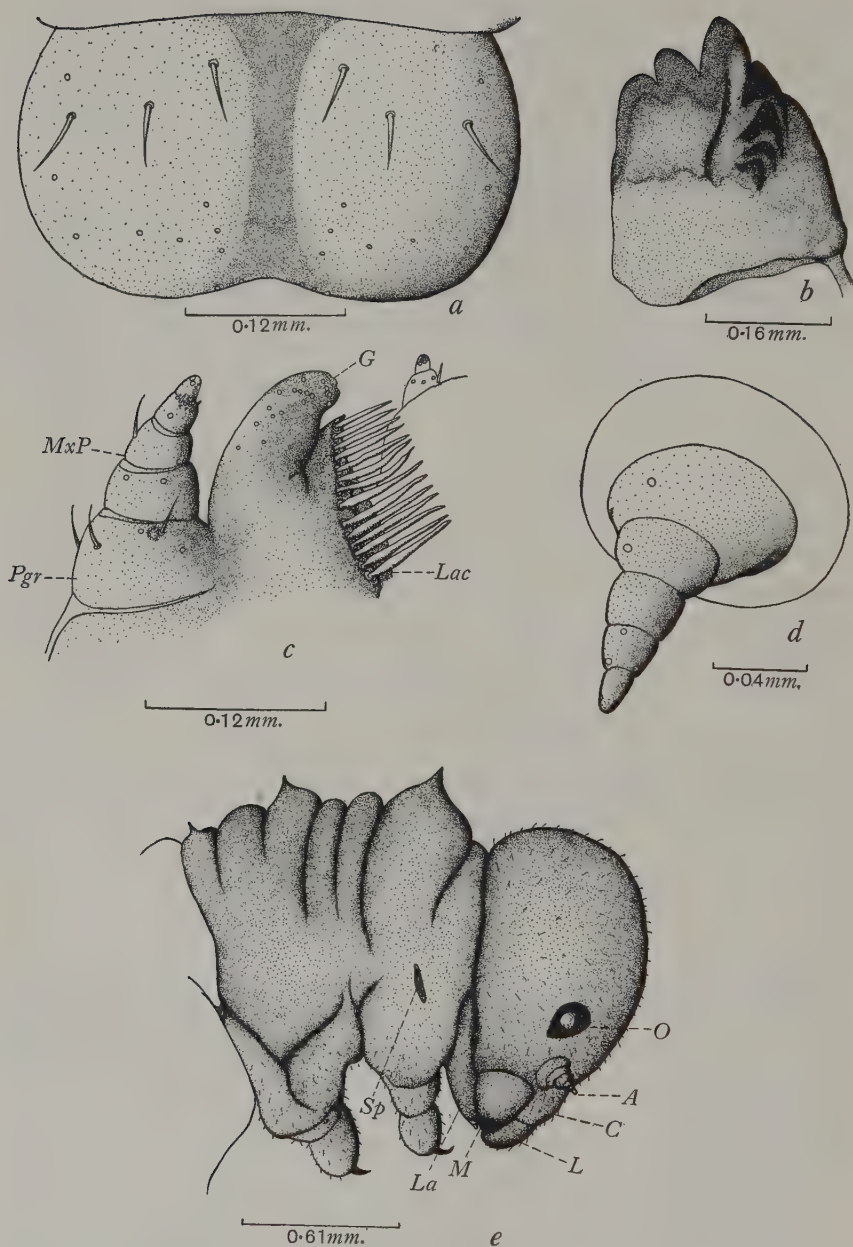
The genus *Endelomyia* Ashmead closely resembles *Caliroa* Costa in both adult and larval characters. Adults of *Endelomyia aethiops* are shining black, 5–6 mm. long and with a wing expanse of 10–11 mm. The wings of both sexes have a slight brownish infuscation throughout, and in the specimens examined there is no tendency towards denser infuscation below the stigma as in *Caliroa*. In the forewing the medio-cubital cross vein is only slightly bent and the radial cross vein almost always strikes the costal margin of cell R 4 equidistant from R 4 and R 5. Cell A 2 is sharply contracted at one-third from the base; A 3 is somewhat thickened at the contraction and a short spur is projected into cell A 2. This is in marked contrast to the evenly emarginate contraction of cell A 2 in *Caliroa*. The hindwing of *E. aethiops* has a single median enclosed cell,

M4 + 1st M2. The vein M3 unites with A1 + 2 some distance from their point of fusion. The anterior and intermediate pairs of legs have the bases of the femora dark and the tips of the femora, the tibiae and the tarsi brownish. In the posterior legs the femora are mainly dark; the tibiae are dark except at their extreme base, and the tarsi are distinctly darker than those of the other legs. The head has the clypeus broadly truncate. The antennae are somewhat shorter and more slender than those of *Caliroa* spp. The second antennal segment is nearly as broad as long but rather shorter and narrower than the first, while in *Caliroa* first and second antennal segments are subequal. Males of *E. aethiops* are smaller and more slender than females but colour and wing venation are the same in both sexes.

Oviposition and incubation period. The flight period for this species is from the middle of May to the middle of June. The females oviposit in the young leaves of rose, preferring *Rosa canina* but occasionally heavily infesting cultivated roses. The eggs are laid at the edges of the leaves, usually in the serrations. The site occupied by the egg measures only about 0.75×0.50 mm. and is visible on the under surface of the leaf as a slight oval swelling. Generally the eggs are laid singly and, though five eggs have been found on a leaf, not more than two have occurred on a leaflet. The incubation period varies from 9 to 14 days.

Description of larvae (Text-fig. 3). The larvae emerge on the lower side of the leaves through a curved slit in the eggshell and the leaf epidermis. The newly hatched larvae wander for some time before feeding and finally settle down to feed on either surface of the leaf. At first they eat tiny fragments from the surface. These first feeding sites are roughly circular and are often concentrated near the bases of the leaflets. Later the larvae seem to avoid direct sunlight and feed by day almost entirely on the under sides of the leaves, but at night they may feed on the upper surface. In captivity the larvae fed on whichever leaf surface was away from the light. First instar larvae gnaw at the surface of the leaf tissue but by the time the larvae reach the second stage they devour the entire leaf tissue leaving only the upper epidermis unbroken. In a short time places where the larvae have fed dry, turn yellow and then brown and the leaves assume a mottled and scorched appearance.

In Cheshire larvae have been found about rose bushes from June 10th to July 14th. The feeding period of larvae under observation was 20–27 days and during that time there were five or six moults, probably depending on sex. Table III gives the number and duration of the larval stadia.



Text-fig. 3. Details of larva of *Endelomyia aethiops*. *a*, dorsal view of labrum. *b*, right mandible. *c*, left maxilla. *d*, antenna. *e*, head and part thorax; *Sp*=spiracle (other lettering as in Text-fig. 2*e*).

Table III.

Larva No. 1	Date of hatching	Duration of larval stadia (in days)					Total in days
		1st	2nd	3rd	4th	5th	
2	June 11	4	3	8	6	—	21
3	"	3	4	4	7	7	25
4	"	3	5	4	10	—	22
5	"	3	4	5	9	—	21
6	"	3	4	7	9	—	23
7	June 12	3	3	7	9	—	22
8	"	3	3	10	5	6	27
9	"	4	2	4	10	—	20
10	"	4	3	6	7	—	20
	"	5	2	7	6	7	27

In the course of breeding experiments observations were made on the rate of growth in the larvae. Measurements were taken of the frons and head capsules of successive larval instars and it was found by calculation that the growth ratio of the head capsules was 1.23, approximately the same as for *C. limacina* and *C. annulipes*.

Mature larvae of *E. aethiops* measure 9–11 mm. Their general colour is yellow but during the feeding period the green contents of the alimentary canal can be seen through the integument. The head is yellow to orange yellow and is clothed with short pale setae. The antennae are prominent and 5-segmented and the eyes and trophi conspicuously dark brown. The thorax is distinctly thicker than the rest of the body but not greatly enlarged as in *Caliroa* larvae. The thoracic legs are short, stout and yellow, with brown tarsal claws. The prolegs are short and pale and occur on segments 5–11 inclusive and also on the caudal segment, those on segment 13 being greatly reduced. The whole body is closely and finely annulated, six annulets being constant for segments 4–10. Larvae of *E. aethiops* differ from larvae of *C. limacina* and *C. annulipes* in having no coating of slime, no conical glands arising near the bases of the prothoracic legs, and in possessing caudal prolegs on segment 13.

Pupation. Larvae under observation completed their development during July 1st–9th. After ceasing to feed the larvae moulted and almost immediately afterwards entered the soil to make their cocoons. They penetrate to some depth and then hollow out an oval cell with the sides made compact by pressure and the soil particles lightly cemented with saliva. These cells are very fragile and when exposed become brittle and easily broken. Within the cocoon the larvae contract and become dense creamy white with the integument closely wrinkled. They hibernate in this condition and pupation takes place in the following spring.

Annual cycle. *E. aethiops* is univoltine in England. Cameron's observations⁽³⁾ indicate that it exhibits the phenomenon of delayed

development noted in certain species of *Hoplocampa* (19). He records that larvae taken by Mr J. E. Fletcher of Worcester remained for 18 months in the cocoons before emerging, and that larvae under his own observations remained two years before completing their development.

Males of *E. aethiops* do not appear to be common. There are only two specimens, from Thornhill and Kilmarnock, in the Cameron collection at the British Museum. On June 2nd, 1934, in south Westmorland, the writer took two males flying above a bush of *Rosa canina* that had been infested by larvae of *E. aethiops* in 1933. In 1934 no larvae were found on the bush and no females were taken about the bush or near it. It is interesting to note that Chittenden (6), writing of this species in America, states, "The males...are quite lively, flying from one bush to another and hovering round their less active partners." Enslin (9), however, observes that males are rare and that the species appears to depend on parthenogenetic reproduction.

Host plants. *E. aethiops* is much more specialised in its feeding habits than *Caliroa* sp. In England it breeds on *Rosa canina*, *R. arvensis*, and on many cultivated varieties of roses. Infestation of cultivated roses often starts on suckers from the stocks and only later do the larvae travel upwards to feed on flowering shoots. There appear to be no records of *E. aethiops* occurring on any host other than *Rosa* sp.

IDENTIFICATION OF *CALIROA* AND *ENDELOMYIA*.

The American entomologist Ashmead separated the genus *Endelomyia* Ashm. from *Caliroa* Costa on the relation between the first and second segments of the antennae and the shape of the clypeus (2). Examination of specimens indicate that the shape of the clypeus is not constant in *Caliroa*, *C. cinxia* Klug. having the clypeus truncate while other species have the clypeus emarginate. It was noted, however, that the shape of cell A2 in the forewing differed in the genera and was constant in the species. In *Caliroa* sp. cell A2 has an even emargination along the anal edge, while in *Endelomyia* the emargination is asymmetrical and a short spur from vein A3 projects into the cell. Rohwer (21, 22) considers that the differences between these two groups are hardly of generic importance and suggests that *Caliroa* Costa and *Endelomyia* Ashmead be considered as sub-genera. The separation of *Endelomyia* from *Caliroa*, however, appears justified on larval characters. Larvae of *E. aethiops* lack the prothoracic glands and slimy coating characteristic of larvae of *Caliroa* sp. and have the thorax less enlarged and the head less retracted. They also

possess a pair of reduced prolegs at the tip of the abdomen, while in *Caliroa* these are lacking.

The species comprising the genera *Caliroa* and *Endelomyia* are similar in size and appearance. They are shining black insects with short stout antennae and often with dusky wings. There are five British species, three of which are common and widely distributed. They can be separated by means of the following key. In the key and throughout the paper the nomenclature of the veins is that given by MacGillivray (14).

Key to Species of Caliroa Costa and Endelomyia Ashmead.

1. Cell A2 of forewing not evenly emarginate on its anal edge and containing short spur from vein A3. Second segment of antenna shorter and narrower than first **E. aethiops** Fab.
Cell A2 of forewing with even emargination on its anal edge. First and second segments of antenna sub-equal (Genus **Caliroa**) **2.**
2. Clypeus truncate **C. cinxia** Klug.
Clypeus emarginate **3.**
3. Posterior tibiae entirely black **C. limacina** Retz.
Posterior tibiae with at least the base whitish **4.**
4. Clypeus deeply emarginate. Wings deeply infuscated on basal two-thirds; apical third almost hyaline. Radial cross vein and R4 distant. Male without continuous external neuration in hindwing. Female with posterior tibiae with at least basal third whitish and posterior metatarsal segments with at least half whitish **C. annulipes** Klug.
5. Clypeus shallowly emarginate. Wings hyaline except for faint infuscation beneath stigma. Radial cross vein and R4 interstitial or nearly so. Male with continuous external neuration in hindwing. Female with posterior tibiae with less than basal third whitish and posterior metatarsal segments with less than basal half whitish **C. varipes** Klug.

C. cinxia and *C. varipes* are not common. Cameron (4) and others (9, 13) state that in the larval stage these species feed on oak, but neither has been taken by the writer. Adults have been examined through the kindness of Mr R. B. Benson of the British Museum and Mr H. Britten of the Manchester Museum.

SUMMARY.

Larvae of *Caliroa* Costa and *Endelomyia* Ashmead are well known as slugworms and leaf skeletonisers of many cultivated trees and shrubs.

C. limacina Retz. oviposits on the under sides of leaves of plum, pear and cherry and the eggs hatch in 11-14 days. The larvae are covered with dark slime and feed on the upper surface of the leaves. The feeding stage

lasts 18–21 days. The larvae moult and shed their coating of slime before entering the soil to make cocoons. The cocoons are composed mainly of soil particles, lightly cemented with saliva. In the north-west of England some individuals emerge the same season and give rise to a second generation, and others continue in the cocoons until the following year. Reproduction is mainly dependent on parthenogenesis.

C. annulipes Klug. oviposits on the upper surface of the leaves of *Salix*, *Crataegus*, *Prunus*, etc. The incubation period is 13–15 days. The larvae, unlike those of *C. limacina*, are covered with transparent slime and feed on the under side of the leaves. The larval stage lasts 19–22 days and at maturity they lose their coating of slime and enter the soil to construct cocoons. *C. annulipes* is bivoltine in the north of England and the phenomenon of delayed development has not so far been observed.

E. aethiops Fab. appears confined to *Rosa* sp. The flight period is May–June and the females oviposit in the edges of rose leaves, preferring *Rosa canina* and *R. arvensis*. The incubation period is 9–14 days and the larval feeding period 20–27 days. The larvae are yellowish; the thorax is enlarged but not as much as in *Caliroa* sp., and the prothoracic glands and coating of slime are lacking. They feed on the under surfaces of the leaves and at maturity they moult and enter the soil to make cocoons. *E. aethiops* is univoltine and hibernation takes place in the contracted prepupal condition in the cocoon.

Endelomyia Ashm. can be separated from *Caliroa* Costa on antennal characters and wing venation. A key for the identification of the five recorded British species of *Caliroa* and *Endelomyia* is included.

The writer is indebted to Mr R. B. Benson, M.A., and Mr H. Britten, F.R.E.S., for the loan of material from time to time, and to Mr Morris Cohen, M.Sc., for assistance in the preparation of the illustrations.

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Fig. 1.

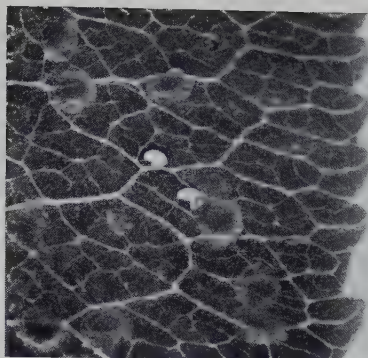


Fig. 2.



Fig. 3.



Fig. 4.



Fig. 5.



Fig. 6.



Fig. 7.

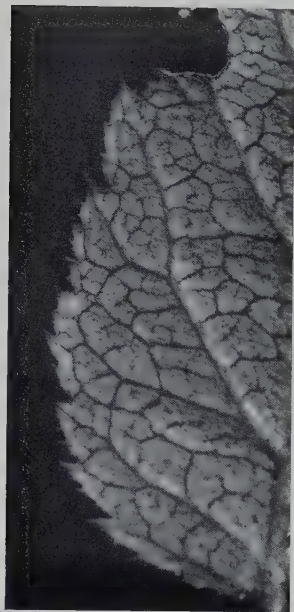


Fig. 8.

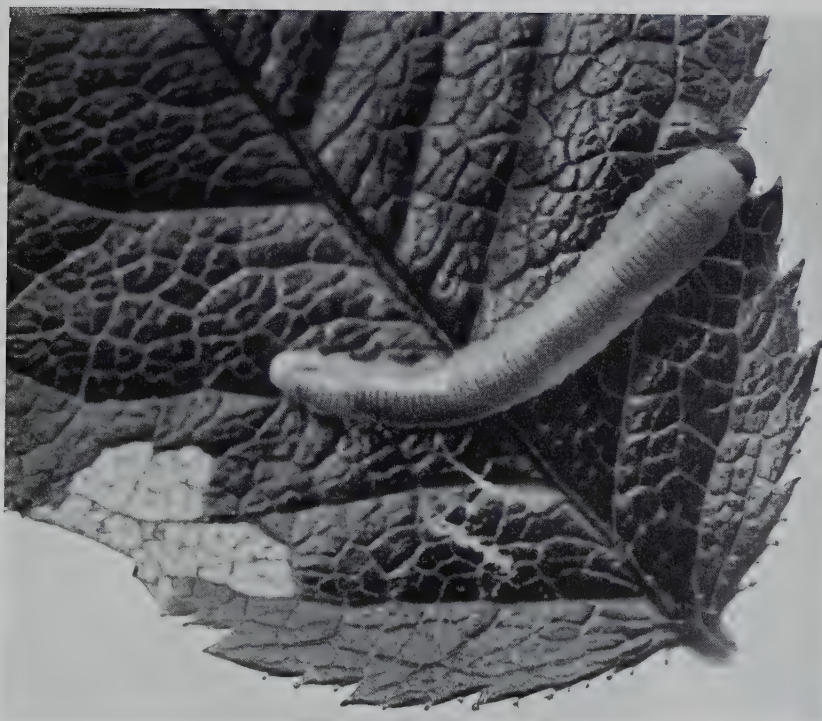


Fig. 9.

MILES.—BIOLOGICAL STUDIES OF CERTAIN SPECIES OF *CALIROA* COSTA AND *ENDELOMYIA* ASHMEAD (HYMENOPTERA SYMPHYTA) (pp. 116-133).

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EXPLANATION OF PLATES XV AND XVI.

PLATE XV.

- Fig. 1. Adults of *C. limacina* (♂ left, ♀ right). × 3.
- Fig. 2. Egg-sites and exposed eggs of *C. limacina*. × 5.
- Fig. 3. Larvae of *C. limacina* on pear leaf. × $\frac{3}{4}$.
- Fig. 4. Larva of *C. limacina* before shedding slimy covering. × 4.
- Fig. 5. Larvae of *C. limacina* after shedding slimy covering. × 4.
- Fig. 6. Larva (prepupa) of *C. limacina* extracted from cocoon. × 10.

PLATE XVI.

- Fig. 7. Adult ♀ *E. aethiops*. × 9.
- Fig. 8. Part of leaflet of rose showing egg of *E. aethiops* inserted in the margin. × 5.
- Fig. 9. Fifth instar larva of *E. aethiops*. × 8.

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THE CALCULATION OF THE DOSAGE-MORTALITY CURVE

By C. I. BLISS.

(*Galton Laboratory, University College, London.*)

(With 3 Text-figures.)

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TOXICOLOGICAL studies upon a large variety of organisms by many biologists have established the sigmoid character of the typical dosage-mortality curve, especially in the case of multicellular forms. Recently it has been shown in two different fields that such curves can easily be plotted as straight lines and their later analysis thereby facilitated (1, 5, 6). These methods, which are substantially the same, are developed more fully in the present paper. While the procedures have been selected on the basis of their statistical accuracy and efficiency, and accordingly follow the recent trends which are so closely associated with the name of R. A. Fisher, an attempt has been made to present them in sufficient detail to permit their use by biologists with a limited knowledge of statistics. The present paper is concerned with the calculation of the transformed dosage-mortality curve and its accuracy. Later papers in this series will deal with statistical methods for comparing dosage-mortality data, and with time-survival curves.

I. THE INTERPRETATION OF THE DOSAGE-MORTALITY CURVE AND ITS TRANSFORMATION TO A STRAIGHT LINE.

Action curves in pharmacology are those in which the amount of the response to any given degree of chemical or physical stimulation is expressed as a percentage of the maximum obtainable in that particular biological system. The action curve is frequently sigmoid, especially when it expresses the relationship of mortality to dosage, so that a graphic plot of the percentage of dead organisms on the ordinate against some function of dosage along the abscissa resembles the letter *S*, the change in percentage kill per unit of the abscissa being smallest near mortalities of 0 and 100 per cent., and largest near 50 per cent. Among multicellular organisms, it is practically universal for a diagram with these co-ordinates to show this characteristic shape, but the interpretation of such curves has varied widely. Since this controversy has been reviewed so fully by Clark (2), the ground need not be gone over again, and we may proceed at once to describe the viewpoint adopted here.

On this theory, the dosage-mortality curve is primarily descriptive of the variation in susceptibility between the individuals of a population. Let us suppose that, under uniform conditions, the susceptibility of each individual may be represented by the smallest dose which is just sufficient to kill it, the individual lethal dose. As in the case of any other biological characteristic, this susceptibility will vary from one individual to another in the population, and *a priori* we might expect the distribution curve of the number of individuals having each particular susceptibility to show the shape characteristic of the normal curve of error. If Fig. 1, which is the normal curve of error in its most usual form, is assumed, for the moment, to be an ideal representation of the variation in susceptibility, the ordinates will give the number of individual organisms corresponding to each particular individual lethal dose shown along the base in a graded series (assuming that the numbers along the base of the figure are equivalent to actual dosages in one form or another).

With intact animals, however, the experimental technique is usually not suitable for determining the exact minimum lethal dose for each individual, as would be required to secure the data for plotting this form of the normal frequency curve of error. As the experiment is actually conducted, the dosage applied to each separate lot of organisms kills not only those requiring at least this quantity of poison, but also all more susceptible individuals, *i.e.* those which could be killed with a smaller

dosage. Consequently, if Fig. 1 represents the hypothetical frequency distribution of susceptibility, as measured by the individual lethal dose, any given dose will split the sample of organisms into two categories of dead and alive, whose relative proportion will depend upon the relation of the dosage to the distribution of susceptibilities. If our dose had happened to come at the point marked x in Fig. 1, the ratio of the dead or more susceptible individuals to the total number in the sample treated—in other words, the percentage killed—would have been the ratio of the unshaded area to the total area under the curve. By varying our dosage

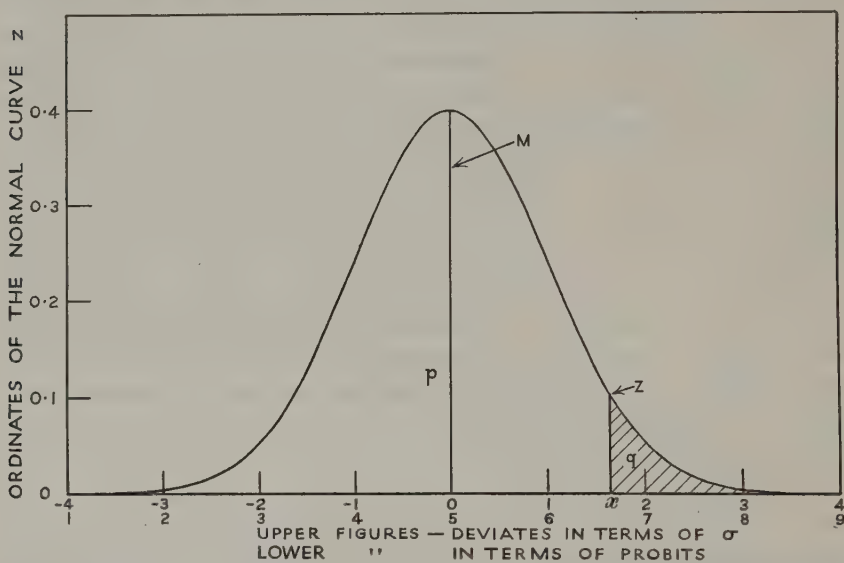


Fig. 1. The theoretical normal curve of error, in which p (0.95) and q (0.05) indicate areas under the curve to the left and right respectively of the ordinate z erected at the point on the abscissa indicated by x (1.645σ). The position of the median (and also of the mean and the mode) is given by M which divides the area under the curve into halves.

along the base and using a succession of equivalent samples of organisms, it would be possible to determine a series of percentage kills (or proportionate areas, $\frac{p}{p+q}$, of the normal frequency curve) corresponding to the dosages applied experimentally. If these percentage kills were then plotted on the ordinate of another graph against the dosage on the abscissa as before, the result would be a cumulative normal frequency distribution such as Fig. 2. This type of curve, therefore, can be and frequently is obtained experimentally in the laboratory.

The assumption that the individual susceptibility to a poison is distributed normally may be tested by reversing our argument. From a given sample of 40 beetles, let us say, exposed to a known concentration of fumigant, 38, or 95 per cent., were killed. Temporarily neglecting the observed dosage, this percentage kill may be equated to a fraction of the total area under the theoretical normal curve of error, $\frac{p}{p+q}$, and the "expected" dosage, x , to which this mortality corresponds, read from the

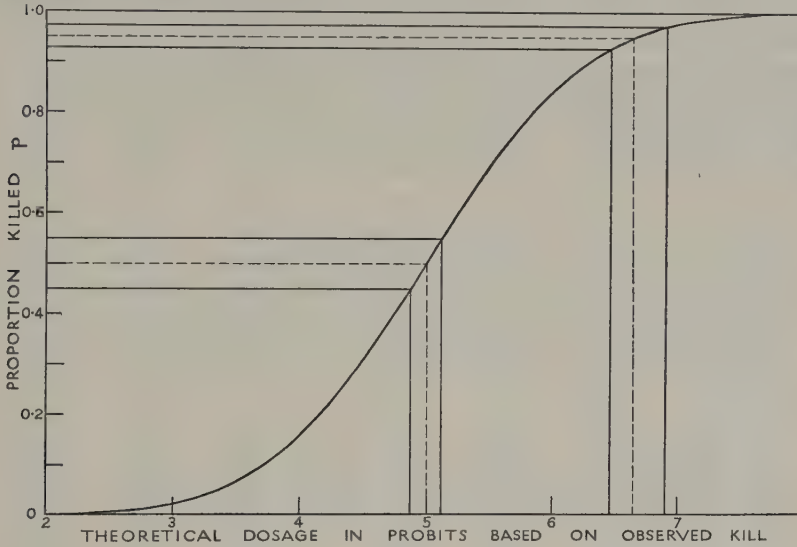


Fig. 2. The proportionate areas, $\frac{p}{p+q}$, of Fig. 1 plotted on the same abscissa as before (probit units). The "broken" lines are drawn at the same positions as the two ordinates, M and xz , of Fig. 1, while the solid parallel lines bounding the broken lines mark the corresponding limits of the standard error for a sample of 100 individuals.

base (Fig. 1). Because of the availability of statistical tables, this expected dosage is given most conveniently in units of standard deviations. The standard deviation, σ , corresponding to any observed mortality may be read directly from sources such as the Kelley-Wood Table(7) or the Shepard-Galton Table(9), and in this case would be 1.645 standard deviations. Similarly, another sample of 40 beetles at a lower dosage, may have shown a mortality of only 20 individuals or 50 per cent., and the expected dosage inferred from this mortality would be 0 standard deviations, since the standard deviation in the normal curve is measured from the median or mean as the origin.

In this fashion an expected dosage corresponding to every observed dosage measured experimentally may be determined from the observed mortality, and the inferred dosages, so derived, are called "normal equivalent deviations" or "N.E.D." by Gaddum⁽⁵⁾ and by Hemmingsen⁽⁶⁾. Many observations, however, will fall below 50 per cent. kill and by Gaddum's system would require negative expected dosages, which are inconvenient. In order to avoid this difficulty, a new table of statistical units called "probits" has been devised⁽¹⁾ in which the 0 of the usual statistical table of deviates has been equated to the digit 5, and the deviate of the normal curve, in terms of σ , added algebraically to secure the probit corresponding to each percentage kill (Table I). Because of their greater convenience, the expected dosages may be expressed in terms of probits and will not modify the proof or disproof of our basic assumption.

Table I.

Probits or probability units for transforming the sigmoid dosage-mortality curve to a straight line. In the body of the table is given the probit corresponding to each percentage mortality listed along the left edge and top.

	0-0	0-1	0-2	0-3	0-4	0-5	0-6	0-7	0-8	0-9
0	—	1-9098	2-1218	2-2522	2-3479	2-4242	2-4879	2-5427	2-5911	2-6344
1	2-6737	2-7096	2-7429	2-7738	2-8027	2-8299	2-8556	2-8799	2-9031	2-9251
2	2-9463	2-9665	2-9859	3-0046	3-0226	3-0400	3-0569	3-0732	3-0890	3-1043
3	3-1192	3-1337	3-1478	3-1616	3-1750	3-1881	3-2009	3-2134	3-2256	3-2376
4	3-2493	3-2608	3-2721	3-2831	3-2940	3-3046	3-3151	3-3253	3-3354	3-3454
5	3-3551	3-3648	3-3742	3-3836	3-3928	3-4018	3-4107	3-4195	3-4282	3-4368
6	3-4452	3-4536	3-4618	3-4699	3-4780	3-4859	3-4937	3-5015	3-5091	3-5167
7	3-5242	3-5316	3-5389	3-5462	3-5534	3-5605	3-5675	3-5745	3-5813	3-5882
8	3-5949	3-6016	3-6083	3-6148	3-6213	3-6278	3-6342	3-6405	3-6468	3-6531
9	3-6592	3-6654	3-6715	3-6775	3-6835	3-6894	3-6953	3-7012	3-7070	3-7127
10	3-7184	3-7241	3-7298	3-7354	3-7409	3-7464	3-7519	3-7574	3-7628	3-7681
11	3-7735	3-7788	3-7840	3-7893	3-7945	3-7996	3-8048	3-8099	3-8150	3-8200
12	3-8250	3-8300	3-8350	3-8399	3-8448	3-8497	3-8545	3-8593	3-8641	3-8689
13	3-8736	3-8783	3-8830	3-8877	3-8923	3-8969	3-9015	3-9061	3-9107	3-9152
14	3-9197	3-9242	3-9286	3-9331	3-9375	3-9419	3-9463	3-9506	3-9550	3-9593
15	3-9636	3-9678	3-9721	3-9763	3-9806	3-9848	3-9890	3-9931	3-9973	4-0014
16	4-0055	4-0096	4-0137	4-0178	4-0218	4-0259	4-0299	4-0339	4-0379	4-0419
17	4-0458	4-0498	4-0537	4-0576	4-0615	4-0654	4-0693	4-0731	4-0770	4-0808
18	4-0846	4-0884	4-0922	4-0960	4-0998	4-1035	4-1073	4-1110	4-1147	4-1184
19	4-1221	4-1258	4-1295	4-1331	4-1367	4-1404	4-1440	4-1476	4-1512	4-1548
20	4-1584	4-1619	4-1655	4-1690	4-1726	4-1761	4-1796	4-1831	4-1866	4-1901
21	4-1936	4-1970	4-2005	4-2039	4-2074	4-2108	4-2142	4-2176	4-2210	4-2244
22	4-2278	4-2312	4-2345	4-2379	4-2412	4-2446	4-2479	4-2512	4-2546	4-2579
23	4-2612	4-2644	4-2677	4-2710	4-2743	4-2775	4-2808	4-2840	4-2872	4-2905
24	4-2937	4-2969	4-3001	4-3033	4-3065	4-3097	4-3129	4-3160	4-3192	4-3224

Table I (*cont.*).

	0·0	0·1	0·2	0·3	0·4	0·5	0·6	0·7	0·8	0·9
25	4·3255	4·3287	4·3318	4·3349	4·3380	4·3412	4·3443	4·3474	4·3505	4·3536
26	4·3567	4·3597	4·3628	4·3659	4·3689	4·3720	4·3750	4·3781	4·3811	4·3842
27	4·3872	4·3902	4·3932	4·3962	4·3992	4·4022	4·4052	4·4082	4·4112	4·4142
28	4·4172	4·4201	4·4231	4·4260	4·4290	4·4319	4·4349	4·4378	4·4408	4·4437
29	4·4466	4·4495	4·4524	4·4554	4·4583	4·4612	4·4641	4·4670	4·4698	4·4727
30	4·4756	4·4785	4·4813	4·4842	4·4871	4·4899	4·4928	4·4956	4·4985	4·5013
31	4·5041	4·5070	4·5098	4·5126	4·5155	4·5183	4·5211	4·5239	4·5267	4·5295
32	4·5323	4·5351	4·5379	4·5407	4·5435	4·5462	4·5490	4·5518	4·5546	4·5573
33	4·5601	4·5628	4·5656	4·5684	4·5711	4·5739	4·5766	4·5793	4·5821	4·5848
34	4·5875	4·5903	4·5930	4·5957	4·5984	4·6011	4·6039	4·6066	4·6093	4·6120
35	4·6147	4·6174	4·6201	4·6228	4·6255	4·6281	4·6308	4·6335	4·6362	4·6389
36	4·6415	4·6442	4·6469	4·6495	4·6522	4·6549	4·6575	4·6602	4·6628	4·6655
37	4·6681	4·6708	4·6734	4·6761	4·6787	4·6814	4·6840	4·6866	4·6893	4·6919
38	4·6945	4·6971	4·6998	4·7024	4·7050	4·7076	4·7102	4·7129	4·7155	4·7181
39	4·7207	4·7233	4·7259	4·7285	4·7311	4·7337	4·7363	4·7389	4·7415	4·7441
40	4·7467	4·7492	4·7518	4·7544	4·7570	4·7596	4·7622	4·7647	4·7673	4·7699
41	4·7725	4·7750	4·7776	4·7802	4·7827	4·7853	4·7879	4·7904	4·7930	4·7955
42	4·7981	4·8007	4·8032	4·8058	4·8083	4·8109	4·8134	4·8160	4·8185	4·8211
43	4·8236	4·8262	4·8287	4·8313	4·8338	4·8363	4·8389	4·8414	4·8440	4·8465
44	4·8490	4·8516	4·8541	4·8566	4·8592	4·8617	4·8642	4·8668	4·8693	4·8718
45	4·8743	4·8769	4·8794	4·8819	4·8844	4·8870	4·8895	4·8920	4·8945	4·8970
46	4·8996	4·9021	4·9046	4·9071	4·9096	4·9122	4·9147	4·9172	4·9197	4·9222
47	4·9247	4·9272	4·9298	4·9323	4·9348	4·9373	4·9398	4·9423	4·9448	4·9473
48	4·9498	4·9524	4·9549	4·9574	4·9599	4·9624	4·9649	4·9674	4·9699	4·9724
49	4·9749	4·9774	4·9799	4·9825	4·9850	4·9875	4·9900	4·9925	4·9950	4·9975
50	5·0000	5·0025	5·0050	5·0075	5·0100	5·0125	5·0150	5·0175	5·0201	5·0226
51	5·0251	5·0276	5·0301	5·0326	5·0351	5·0376	5·0401	5·0426	5·0451	5·0476
52	5·0502	5·0527	5·0552	5·0577	5·0602	5·0627	5·0652	5·0677	5·0702	5·0728
53	5·0753	5·0778	5·0803	5·0828	5·0853	5·0878	5·0904	5·0929	5·0954	5·0979
54	5·1004	5·1030	5·1055	5·1080	5·1105	5·1130	5·1156	5·1181	5·1206	5·1231
55	5·1257	5·1282	5·1307	5·1332	5·1358	5·1383	5·1408	5·1434	5·1459	5·1484
56	5·1510	5·1535	5·1560	5·1586	5·1611	5·1637	5·1662	5·1687	5·1713	5·1738
57	5·1764	5·1789	5·1815	5·1840	5·1866	5·1891	5·1917	5·1942	5·1968	5·1993
58	5·2019	5·2045	5·2070	5·2096	5·2121	5·2147	5·2173	5·2198	5·2224	5·2250
59	5·2275	5·2301	5·2327	5·2353	5·2378	5·2404	5·2430	5·2456	5·2482	5·2508
60	5·2533	5·2559	5·2585	5·2611	5·2637	5·2663	5·2689	5·2715	5·2741	5·2767
61	5·2793	5·2819	5·2845	5·2871	5·2898	5·2924	5·2950	5·2976	5·3002	5·3029
62	5·3055	5·3081	5·3107	5·3134	5·3160	5·3186	5·3213	5·3239	5·3266	5·3292
63	5·3319	5·3345	5·3372	5·3398	5·3425	5·3451	5·3478	5·3505	5·3531	5·3558
64	5·3585	5·3611	5·3638	5·3665	5·3692	5·3719	5·3745	5·3772	5·3799	5·3826
65	5·3853	5·3880	5·3907	5·3934	5·3961	5·3989	5·4016	5·4043	5·4070	5·4097
66	5·4125	5·4152	5·4179	5·4207	5·4234	5·4261	5·4289	5·4316	5·4344	5·4372
67	5·4399	5·4427	5·4454	5·4482	5·4510	5·4538	5·4565	5·4593	5·4621	5·4649
68	5·4677	5·4705	5·4733	5·4761	5·4789	5·4817	5·4845	5·4874	5·4902	5·4930
69	5·4959	5·4987	5·5015	5·5044	5·5072	5·5101	5·5129	5·5158	5·5187	5·5215
70	5·5244	5·5273	5·5302	5·5330	5·5359	5·5388	5·5417	5·5446	5·5476	5·5505
71	5·5534	5·5563	5·5592	5·5622	5·5651	5·5681	5·5710	5·5740	5·5769	5·5799
72	5·5828	5·5858	5·5888	5·5918	5·5948	5·5978	5·6008	5·6038	5·6068	5·6098
73	5·6128	5·6158	5·6189	5·6219	5·6250	5·6280	5·6311	5·6341	5·6372	5·6403
74	5·6433	5·6464	5·6495	5·6526	5·6557	5·6588	5·6620	5·6651	5·6682	5·6713

Table I (cont.).

	0-0	0-1	0-2	0-3	0-4	0-5	0-6	0-7	0-8	0-9
75	5-6745	5-6776	5-6808	5-6840	5-6871	5-6903	5-6935	5-6967	5-6999	5-7031
76	5-7063	5-7095	5-7128	5-7160	5-7192	5-7225	5-7257	5-7290	5-7323	5-7356
77	5-7388	5-7421	5-7454	5-7488	5-7521	5-7554	5-7588	5-7621	5-7655	5-7688
78	5-7722	5-7756	5-7790	5-7824	5-7858	5-7892	5-7926	5-7961	5-7995	5-8030
79	5-8064	5-8099	5-8134	5-8169	5-8204	5-8239	5-8274	5-8310	5-8345	5-8381
80	5-8416	5-8452	5-8488	5-8524	5-8560	5-8596	5-8633	5-8669	5-8705	5-8742
81	5-8779	5-8816	5-8853	5-8890	5-8927	5-8965	5-9002	5-9040	5-9078	5-9116
82	5-9154	5-9192	5-9230	5-9269	5-9307	5-9346	5-9385	5-9424	5-9463	5-9502
83	5-9542	5-9581	5-9621	5-9661	5-9701	5-9741	5-9782	5-9822	5-9863	5-9904
84	5-9945	5-9986	6-0027	6-0069	6-0110	6-0152	6-0194	6-0237	6-0279	6-0322
85	6-0364	6-0407	6-0450	6-0494	6-0537	6-0581	6-0625	6-0669	6-0714	6-0758
86	6-0803	6-0848	6-0893	6-0939	6-0985	6-1031	6-1077	6-1123	6-1170	6-1217
87	6-1264	6-1311	6-1359	6-1407	6-1455	6-1503	6-1552	6-1601	6-1650	6-1700
88	6-1750	6-1800	6-1850	6-1901	6-1952	6-2004	6-2055	6-2107	6-2160	6-2212
89	6-2265	6-2319	6-2372	6-2426	6-2481	6-2536	6-2591	6-2646	6-2702	6-2759
90	6-2816	6-2873	6-2930	6-2988	6-3047	6-3106	6-3165	6-3225	6-3285	6-3346
91	6-3408	6-3469	6-3532	6-3595	6-3658	6-3722	6-3787	6-3852	6-3917	6-3984
92	6-4051	6-4118	6-4187	6-4255	6-4325	6-4395	6-4466	6-4538	6-4611	6-4684
93	6-4758	6-4833	6-4909	6-4985	6-5063	6-5141	6-5220	6-5301	6-5382	6-5464
94	6-5548	6-5632	6-5718	6-5805	6-5893	6-5982	6-6072	6-6164	6-6258	6-6352
95	6-6449	6-6546	6-6646	6-6747	6-6849	6-6954	6-7060	6-7169	6-7279	6-7392
96	6-7507	6-7624	6-7744	6-7866	6-7991	6-8119	6-8250	6-8384	6-8522	6-8663
97	6-8808	6-8957	6-9110	6-9268	6-9431	6-9600	6-9774	6-9954	7-0141	7-0335
98-0	7-0537	7-0558	7-0579	7-0600	7-0621	7-0642	7-0663	7-0684	7-0706	7-0727
98-1	7-0749	7-0770	7-0792	7-0814	7-0836	7-0858	7-0880	7-0902	7-0924	7-0947
98-2	7-0969	7-0992	7-1015	7-1038	7-1060	7-1084	7-1107	7-1130	7-1154	7-1177
98-3	7-1201	7-1224	7-1248	7-1272	7-1297	7-1321	7-1345	7-1370	7-1394	7-1419
98-4	7-1444	7-1469	7-1494	7-1520	7-1545	7-1571	7-1596	7-1622	7-1648	7-1675
98-5	7-1701	7-1727	7-1754	7-1781	7-1808	7-1835	7-1862	7-1890	7-1917	7-1945
98-6	7-1973	7-2001	7-2029	7-2058	7-2086	7-2115	7-2144	7-2173	7-2203	7-2232
98-7	7-2262	7-2292	7-2322	7-2353	7-2383	7-2414	7-2445	7-2476	7-2508	7-2539
98-8	7-2571	7-2603	7-2636	7-2668	7-2701	7-2734	7-2768	7-2801	7-2835	7-2869
98-9	7-2904	7-2938	7-2973	7-3009	7-3044	7-3080	7-3116	7-3152	7-3189	7-3226
99-0	7-3263	7-3301	7-3339	7-3378	7-3416	7-3455	7-3495	7-3535	7-3575	7-3615
99-1	7-3656	7-3698	7-3739	7-3781	7-3824	7-3867	7-3911	7-3954	7-3999	7-4044
99-2	7-4089	7-4135	7-4181	7-4228	7-4276	7-4324	7-4372	7-4422	7-4471	7-4522
99-3	7-4573	7-4624	7-4677	7-4730	7-4783	7-4838	7-4893	7-4949	7-5005	7-5063
99-4	7-5121	7-5181	7-5241	7-5302	7-5364	7-5427	7-5491	7-5556	7-5622	7-5690
99-5	7-5758	7-5828	7-5899	7-5972	7-6045	7-6121	7-6197	7-6276	7-6356	7-6437
99-6	7-6521	7-6606	7-6693	7-6783	7-6874	7-6968	7-7065	7-7164	7-7265	7-7370
99-7	7-7478	7-7589	7-7703	7-7821	7-7944	7-8070	7-8202	7-8338	7-8480	7-8627
99-8	7-8782	7-8943	7-9112	7-9291	7-9478	7-9677	8-9889	7-0114	8-1357	8-0618
99-9	8-0902	8-1214	8-1559	8-1947	8-2389	8-2905	8-3528	8-4316	8-5401	8-7190

The next step is to plot on the ordinate the probit of the expected dosage, inferred from the observed mortality, and on the abscissa some function of the amounts which were administered experimentally. These latter may be originally in terms of the concentrations of a toxic

substance in which the successive lots of organisms were immersed for a given time, a graded series of times of exposure to a fixed concentration of poison, doses administered individually at different units per gram of body weight, different concentrations of contact poison applied uniformly over the surface of the body, or in some other terms. When these units of measurement are plotted directly, the resulting curve is very seldom a straight line but is nearly always convex upwards, an effect which might have been anticipated from the markedly asymmetrical character of most sigmoid dosage-mortality curves.

Before discarding the normal curve as an adequate description of the variation between individuals in their susceptibility to a poison, let us question the assumption that the individual lethal dose is a satisfactory direct measure of susceptibility. The dosage units described above form an arithmetical scale of equal increments, and would not be a satisfactory index to the susceptibility if the structural or chemical constituents which determine the level of susceptibility of the individual in respect to a given drug were not to increase or decrease by equal additive increments. It was pointed out as long ago as 1879 by Galton that in biological material the variation often shows a geometrical rather than an arithmetical distribution, an observation which has been confirmed by several investigators in respect to toxicological characteristics. If, therefore, the changes in the substances or structures which determine susceptibility, whatever may be their nature, were ordinarily proportional in type, then they would be symmetrically distributed not on an arithmetical scale of individual lethal doses but only on a logarithmic scale. This possibility may be tested by converting the observed dosages to logarithms and again plotting the dosages inferred from mortality or probits against those secured experimentally. With this transformation, a straight line does result in a great majority of the cases which have been tested. Before the method of inferring "expected" doses from the percentage kills had been devised, Trevan⁽¹³⁾ and others had shown that per cent. mortality plotted against the logarithm of the dose frequently results in symmetrical sigmoid curves, while in the descriptions (1, 5, 6) of the double transformation, many more cases were cited in which the logarithm of the individual dose was an adequate measure of susceptibility.

If the transformation of dosages to logarithms completes the transformation of the dosage-mortality curve to a straight line because it is an index to the inherent susceptibility of the individual animal to the poison, the poisoning process could be considered as an example of the Weber-Fechner law. This implies, however, a direct proportionality

between the concentration of the poison in the dose administered and the amount of poison fixed by the essential tissues of the animal, and there is no evidence in support of such a direct relationship. Moreover, if the poisoning of the individual multicellular animal can be attributed to the death of a certain proportion of its cells, then the susceptibility of the animal as a whole will be determined by the average susceptibility of its essential cells. Even though the susceptibility of these ultimate units, the cells, may vary geometrically rather than arithmetically, so that their distribution is highly asymmetrical, it is probable that the average susceptibilities of populations of these unit cells, the individual animals, are symmetrically and normally distributed, if we may judge from general statistical experience. *A priori*, therefore, the individual animals in a stock may be expected to vary normally in their susceptibility to a specific poison, since each animal is an "average" of its component cells. The justification of the logarithmic transformation may be sought in the relation between the dosage administered and the amount of poison fixed by the essential cells or tissues, rather than in the Weber-Fechner law.

The fixation of a drug or poison seems to be primarily a phenomenon of adsorption(2), and one of the two principal formulae for describing this process is that proposed by Freundlich. Freundlich's empirical formula is

$$KC_n^{\frac{1}{m}} = \frac{x}{m},$$

where, for our purposes, C may be equated to the concentration of the drug (or dosage), x =the amount fixed in the organism, m =the mass of adsorbing constituents within the organism, and K and n are constants. If the variation in susceptibility is attributed primarily to the reactions which follow the fixation of the poison, m will be constant from one individual test animal to the next. By combining constants, the Freundlich formula may be reduced to

$$\log C = n \log x + K',$$

from which it is apparent that there is a linear relation between the logarithm of the concentration (or dosage) and the logarithm of the amount fixed by the cells of the animal. The observed logarithmic conversion of the dosage-mortality curve is not due, therefore, to our using as the true individual lethal dose the amount fixed in the tissue, if this is related to the concentration by the Freundlich formula.

In many instances another adsorption equation, that proposed by Langmuir, has fitted the biological data on the fixation of drugs more satisfactorily than the Freundlich formula. Moreover, it is better

grounded theoretically. Langmuir's adsorption equation is given by Clark as

$$kx^n = \frac{y}{100-y},$$

where x =concentration of the drug, y =percentage of the maximum amount of drug which can be fixed by the cell, n is determined by the molecular state of the fixed drug as compared with its state before adsorption and is usually 1 or 2, and k is a constant. In order to compare the amount (percentage) fixed with the logarithm of dosage (y with $\log x$), y was calculated for each of a series of hypothetical values of x when $k=0.0625$ and $n=1$. A diagram of y against $\log x$ gave a sigmoid curve, symmetrical about 50 per cent. fixation, and very nearly a straight line between 20 and 80 per cent. fixation. If 100 per cent. kill on the dosage-mortality curve were to correspond to 100 per cent. fixation of the poison by the tissues of the experimental animals, all cases in which the logarithm-probit plot showed a straight line over a range of dosages that included kills of 90 per cent. and better—as very many of them do—would definitely rule out the Langmuir adsorption equation as an explanation. However, investigations have shown that live tissue is capable of adsorbing much more of the chemical than the amount which produces the maximum effect, in this case, the subsequent death of all individuals. If all experimental animals were to die before a dosage is reached which produces 80 per cent. or more adsorption, the logarithm-probit transformation would still be consistent with an interpretation based on the Langmuir adsorption equation, so far as the middle and higher kills—and dosages—are concerned.

The application of the Langmuir equation to the lower dosages presents a more involved problem. Usually the logarithm-probit plot of the dosage-mortality curve can be fitted by a single straight line over the entire range of mortalities, and it may then be reasonable to assume that the amount of poison fixed must exceed a threshold value of 20 per cent. of the maximum before even the most susceptible individuals will be killed. However, in many cases the transformed dosage-mortality line agrees with the higher kills very satisfactorily but indicates too small a mortality below 20 to 35 per cent. kill. At its lower end the otherwise straight line would need to bend up if it is to fit the entire range of observations. The similarity of this change in slope to the lower end of the theoretical curve secured by plotting the percentage of drug fixed against the logarithm of dosage suggests that in these cases the adsorption is less than 20 per cent. of the maximum at the threshold concentration of the

poison, and that if the observed dosage could be converted to the amount fixed by means of the Langmuir equation, a single straight line would be obtained by the use of probits.

Without measurements of the amount of poison adsorbed, the Langmuir equation cannot be tested critically, but an approximate graphic analysis has been applied successfully to several series of fumigation tests in which at the lower dosages there was a change of slope upon the logarithm-probit co-ordinates. For each series of points, the mortality in probits could be fitted satisfactorily (as in Fig. 3) with two intersecting straight lines when plotted against the logarithm of the concentration of the fumigant, the bend between the two lines being acute enough for there to be no hesitation in deciding which observations should be grouped. From a graphic comparison with the theoretical curve mentioned above (percentage fixed *v.* log. dosage) of the angle at which these two lines intersected, the observed concentrations were converted to terms of the percentages of maximum adsorption, and when the observed mortalities in probits were replotted against these theoretical dosage units, the data for each poison could be fitted adequately by a single straight line. This transformation of dosage to per cent. adsorbed introduces two additional constants, one attributable to the maximum adsorption which produces no lethal effect and the other to the minimum adsorption which is invariably fatal. On mathematical grounds alone, therefore, the agreement between observations and fitted curve should be as good as when two intersecting straight lines, also involving four constants, are fitted to the same data.

The use of the Langmuir equation need not necessarily eliminate the change in slope that is observed on occasion at the lower dosages upon the logarithm-probit plot. If a minimum of 15 to 20 per cent. adsorption were required to effect a kill, for example, the rectilinearity in the main portion of the curve and the change in slope at its lower end would be the same whether log. dosage or per cent. of maximum adsorption were plotted along the base. Since there is good experimental evidence, as in the case of protective stupefaction with hydrocyanic acid (10), that low concentrations frequently have an action qualitatively different from that of the higher dosages, the change in slope may very well have a biological reality and not be merely a mathematical artifact. Clark¹ thinks that "this break is a fairly common phenomenon. It suggests to me that the characteristic curve besides measuring individual variation also is affected by some relationship between concentration and amount of

¹ Personal communication.

action." Since without another kind of experimental data even an approximate conversion of dosage into percentage adsorption is possible only when there is a change in slope on the logarithm-probit co-ordinates, and may then be of doubtful theoretical significance, it is preferable at present to use the logarithm of the individual lethal dose as a measure of susceptibility with the understanding that its use can be interpreted in terms other than those of the Weber-Fechner law.

The above procedure should not be confused with another fundamentally different application of the Langmuir adsorption equation, which is hyperbolic, to similar data. If dosage is converted to logarithms, the percentage adsorption plotted against it is a sigmoid curve symmetrical about the 50 per cent. point, as has been described, and the percentage mortality plotted against it is a very similar sigmoid curve. In one case, Clark (p. 157) has considered these two measures as if they were identical, or the percentage mortality a direct measure of percentage adsorption. Yet elsewhere he has described experiments which show that adsorption frequently continues after the point is reached which produces maximum effect, and this possibility alone demonstrates that they are distinct¹. Even if certain dosage-mortality data were fitted adequately by this use of the hyperbolic equation, they could still be considered from the "statistical" viewpoint adopted here. The abscissa, the logarithm of the dose, is the same in both methods of transformation, while the ordinate in both may be assumed to represent sigmoid frequency distributions which are experimentally inseparable between kills of 15 and 85 per cent.

¹ In a recent letter to *Nature* (cxxxiv, 323), H. H. Shepard applies an equivalent method to original data that are similar to those quoted here in Table IV, except that he uses the dosage directly instead of the logarithm of the dose. When his data and fitted curve are plotted in a rectilinear form (logarithm of $\frac{\text{per cent. killed}}{\text{per cent. surviving}}$ against concentration), it is apparent that the observed values are still distributed in a sigmoid manner about the straight line, despite his use of the hyperbola. However, when the probit values for percentage mortality are plotted against dosages which have been converted to hypothetical "percentages of poison adsorbed" (by means of the equation $kx^n = \frac{y}{100-y}$), a very satisfactory fit can be obtained with $\log k = -18.2$ and $n = 10.2$. It should be noted that while Shepard used the same species of insect, the same poison, and apparently the same laboratory technique as in the data quoted here from Strand, his results agree in average susceptibility (the median lethal dose), but show a significantly larger range of variability within the population. Shepard apparently has totalled many individual experiments for each dosage, and if, over the period which this required, the average susceptibility in his stock of beetles had fluctuated as much as 10 to 15 per cent., the variability within his population at any one time might well have been consistent with Strand's earlier results which are quoted here.

They differ in mathematical treatment only in that the frequency distribution of susceptibilities in the interpretation followed here is assumed to be normal, while in the hyperbolic interpretation it is that of the z distribution (3).

On the basis of the above assumptions, we may proceed at once to a consideration of how to calculate the best-fitting dosage-mortality curve. The first step is to transform each percentage kill to its probit (Table I) and convert each dosage to its logarithm. The percentage kill will not, however, be the same as the percentage dead if there is an appreciable mortality among the untreated controls or checks. A convenient way of computing the percentage kill in such a case is to multiply the number of individuals used in a particular test by the proportion alive in the untreated controls, which gives the net total of organisms actually exposed to the action of the poison. When the number surviving the treatment is subtracted from this net total, the difference is the number killed, and the number killed (multiplied by 100), divided by the net number exposed is, of course, the percentage killed. The probit, or dosage inferred from mortality, is then plotted on co-ordinate paper against the logarithm of the dosage that was administered experimentally. Inspection of these points with the aid of a straight edge, such as the side of a celluloid triangle, will show very quickly whether they define a straight line over most or the whole of the range of dosages. In cases where the data for the lower dosages seems to be discordant with the straight line that is consistent with the rest of the observations, the straight line is fitted only to the higher dosages. A few cases may occur in which the points seem to be smoothly curvilinear throughout, and in such instances some other function of dosage should be tried which seems to have a toxicological significance. Having determined the range of dosage over which a rectilinear relation seems to hold good, a straight line is drawn through the points.

II. THE PROVISIONAL REGRESSION LINE.

The first estimate of the transformed dosage-mortality curve, which we will call the provisional regression line, is ordinarily not calculated, but represents the best judgment of the experimenter. When the data are consistent, the graphic provisional curve will often come surprisingly close to the corrected curve obtained after computation. Occasionally, however, the observations may be so scattered that the experimenter will prefer to calculate even the provisional regression line. The simplest procedure in this case is to give each experiment a weight of 1 and use

equations (3)–(6) of the next section. In other cases the data may be so uniform that the initial line will serve the needs of the experimenter. Usually, however, the graphic approximation will want correction, and to obtain this corrected curve we compute what is known in statistics as the regression line. The regression line in our case will show the probit which corresponds to any given logarithm of dosage as accurately as this relation can be determined from the experimental data used in its computation.

The provisional regression line serves two essential purposes: it determines what probit values are to be assigned to observed mortalities of 0 and 100 per cent., and it specifies what relative weights are to be given to the separate observations in a series.

(1) *Probit values for 0 and 100 percentage kills.* Although toxicological tests frequently include at one limit small dosages which kill no individuals or at the other limit large dosages which kill all individuals, these values cannot be listed in the standard table of probits (Table I). By means of the provisional regression line, the information in such observations may still be used in determining the corrected regression line. This possibility follows from our basic assumption that the distribution of susceptibility is normal and the fact that while the curve of the normal distribution (Fig. 2) approaches infinitely close to 100 per cent. kill—considering for convenience only the upper limit—it never quite reaches it mathematically at any finite dosage. Within the range of dosages and numbers of organisms ordinarily used in a laboratory test, this mathematical postulate agrees satisfactorily with the biological reality. Thus the smallest dosage giving 100 per cent. kill will be smaller in an experimental series with 30 organisms per dose than in a repetition of the same series using 300 specimens per dose, since in the larger numbers of the second case there is a greater chance of including the less susceptible individuals in each treatment. The mortality in probits that would be expected if we were dealing with very large numbers of organisms is given approximately by an extension of the provisional regression line over the range of these higher dosages. In a note on “The case of zero survivors,” appended to the present paper, R. A. Fisher points out that when the number in the class of survivors is small, the theory of large samples breaks down if applied to the restricted numbers used in toxicological tests. He shows, however, that when zero survivors are observed the probit term for 100 per cent. kill may be derived by the method of maximum likelihood as a difference, which is added to the expected value in probits given by the provisional regression line.

An alternative method for plotting 100 per cent. kills in terms of probits or their equivalents has been proposed by Gaddum⁽⁵⁾. His value is based upon the number of animals exposed to the treatment, but is not used whenever it indicates a smaller mortality than would be expected from the approximate regression line at this dosage. The method proposed here avoids this limitation and is mathematically the more exact.

The procedure to be followed in securing the probit value for 100 per cent. kills may be outlined briefly. The probit given by the extended provisional regression line is read from the graph at the logarithm for the dosage from which none survived. This probit is then entered in column 1 of Table II and the required probit for the observed kill is found in column 3. First differences are given in column 4 for convenience in interpolation if the provisional regression line has been read to 0.01 probit. These values will always fall above the provisional line as would be expected since no survivors were observed, and should be included in computing the corrected curve with a weight determined as described in the next section. The omission of such terms tends to bias the final regression line by exaggerating the number of survivors to be expected.

The same method is available, of course, at the opposite end of the curve, at dosages which fail to kill any individuals, except that the correction in column 2 of Table II is then subtracted from the probit value given by the provisional line. The correction to use in such a case will be that for the probit in column 1 which is as much greater than 5 as the one read from the provisional line is less than 5. These smaller dosages, however, are usually of little interest, and it frequently happens that, below 25 per cent. kill, the regression line which forms an adequate fit above that point is no longer applicable.

(2) *Weights for fitting the regression line.* The reliability of the probit for an observed percentage kill depends not only on how many individuals were counted to determine this percentage but also upon the corresponding probit value of the regression line, or, in actual practice, upon that of the provisional regression line. It is customary to consider the reliability of a percentage as proportional to the number of individuals tested, and the justification for thus weighting by the number of individuals rather than by the square root of the number of individuals is that the reliability of a measure is inversely proportional to the square of its standard error—the variance—and not to the standard error itself. The variance, in turn, is a function not only of the number of cases but also of several other factors, and it is these other factors which it is necessary to take into account. The principle of giving to individual

Table II.

Probit values when 100 per cent. mortality is observed experimentally. The provisional (graphic) dosage-mortality line, based on probits for dosages which were survived by one or more individuals, is extended to cover dosages from which no survivors were observed. The expected probit value indicated by the provisional line at each such dosage is then entered in column 1 and the correction in column 2 is added to it to give the value in probits for 0 survivors (column 3). When the provisional line has been read to 0.01 probits, the first differences in the last column are convenient for interpolation.

Curve value or probit for expected kill	Correction q/z	Probit for observed kill	First differences
5.5	0.8764	6.3764	466
5.6	0.8230	6.4230	519
5.7	0.7749	6.4749	564
5.8	0.7313	6.5313	604
5.9	0.6917	6.5917	640
6.0	0.6557	6.6557	670
6.1	0.6227	6.7227	699
6.2	0.5926	6.7926	723
6.3	0.5649	6.8649	745
6.4	0.5394	6.9394	764
6.5	0.5158	7.0158	782
6.6	0.4940	7.0940	799
6.7	0.4739	7.1739	812
6.8	0.4551	7.2551	825
6.9	0.4376	7.3376	838
7.0	0.4214	7.4214	848
7.1	0.4062	7.5062	857
7.2	0.3919	7.5919	867
7.3	0.3786	7.6786	874
7.4	0.3660	7.7660	883
7.5	0.3543	7.8543	889
7.6	0.3432	7.9432	895
7.7	0.3327	8.0327	901
7.8	0.3228	8.1228	906
7.9	0.3134	8.2134	912
8.0	0.3046	8.3046	916
8.1	0.2962	8.3962	920
8.2	0.2882	8.4882	924
8.3	0.2806	8.5806	928
8.4	0.2734	8.6734	931
8.5	0.2665	8.7665	935
8.6	0.2600	8.8600	938
8.7	0.2538	8.9538	940
8.8	0.2478	9.0478	943
8.9	0.2421	9.1421	

observations weights that are proportional to their statistical reliability follows that described by Thompson⁽¹²⁾ in his analysis of an experiment in sensory discrimination.

The required standard error is shown graphically on the cumulative form of the normal frequency distribution of Fig. 2, in which p , the pro-

portion killed, is plotted on the ordinate against x , the inferred dosage in probits, on the abscissa. The position of the paired horizontal lines cutting the ordinate on either side of 50 and 95 per cent. kill was calculated from the usual formula for the standard error of a proportion, $\sigma = \sqrt{\frac{pq}{N}}$, where p is the proportion killed, $q = 1 - p$, and $N = 100$ individuals exposed to treatment. However, in our transformed dosage-mortality curve, these percentages have been transformed to probits, which are given along the base of the figure, so that the standard error (and variance) which we need is not that for a proportion, p , but that for the corresponding inferred dosage or probit, x , a quantity equivalent to what statisticians call the percentile. From the points of intersection with the curve in Fig. 2 of the standard errors of the proportions (shown by the paired horizontal lines), we will draw paired vertical lines to cut the base at the standard errors of the probits (or percentiles) corresponding to these two proportions of 0.50 and 0.95. While the standard error of p is a maximum at 50 per cent. kill and diminishes toward either 0 or 100 per cent., that of the probit is smallest at 50 per cent. and increases toward either limit. Hence the accuracy of a given probit will increase as it approaches 50 per cent. kill.

The formula for the variance of a percentile is given by Kelley (7) as

$$\frac{\sigma^2 pq}{z^2 N},$$

where σ is the standard deviation, z is the ordinate of the normal curve (see Fig. 1) and is given in tables of the probability integral, and the other terms have their previous significance. This will also be the variance for the probit of a single observed percentage mortality, but since the probit is already in terms of the standard deviation, σ^2 is always equal to 1 and the variance of a probit may be simplified to the form

$$\frac{pq}{Nz^2}.$$

In order, therefore, to give each observation a weight proportional to its true reliability, instead of multiplying it by N , we will multiply by the reciprocal of the variance as our weight, w . Hence

$$w = N \left(\frac{z^2}{pq} \right), \quad \dots\dots(1)$$

where N is the number of organisms exposed to a given dosage of poison and z , p , and q have their previous significance as functions of the normal curve, which, in this case, are fixed by the probit value of the provisional

regression line at the same dosage. The term $\frac{z^2}{pq}$ we will call the weighting coefficient. It has been computed for each 0.1 probit within the useful range of probit values and is given in Table III (column 6). The procedure for determining the correct weights to be used in calculating the corrected regression line is thus made quite easy. After the provisional regression line has been drawn through the plotted points of the experimental series as described, the probit given by this line for the log. dosage used in each determination is read from the graph to the nearest 0.1 (or 0.01) probit and by reference to Table III is transformed directly to the weighting

Table III.

Weighting coefficients used in computing the dosage-mortality curve in terms of probits. The probit for the expected kill is read to the nearest 0.1 or 0.01 from the provisional, graphic dosage-mortality line at the dosage used in a given test. Entering this in column 1 below, the weighting coefficient is read from column 3 (interpolating from the first differences in column 4 if the line has been read to 0.01 probit) and multiplied by the total number of organisms to secure the weight (w) of the test for use in computing the final curve. The weighting coefficients in column 3 have been abbreviated for ease of calculation from the five-place values of z^2/pq in column 6. Column 5 shows the relative number of individuals which must be used at different expected mortalities if all observations are to be weighted equally; while column 2 gives the percentage mortalities corresponding to the probits in column 1.

Curve value or probit for expected kill	Expected percentage kill	Weighting coefficient	First differences	Relative no. of individuals for equal weights	$\frac{z^2}{pq}$
1.5	0.023	0.0033		1947	0.00327
1.6	0.034	0.0045	12	1412	0.00451
1.7	0.048	0.0061	16	1037	0.00614
1.8	0.069	0.0083	22	769	0.00828
1.9	0.097	0.0110	27	577	0.01104
2.0	0.135	0.0146	36	437	0.01457
2.1	0.187	0.0190	44	334	0.01903
2.2	0.256	0.0246	56	259	0.02459
2.3	0.347	0.0314	68	202	0.03143
2.4	0.466	0.0398	84	160	0.03977
2.5	0.621	0.050	102	128	0.04979
2.6	0.820	0.062	12	103	0.06169
2.7	1.072	0.076	14	84	0.07563
2.8	1.390	0.092	16	69	0.09179
2.9	1.786	0.110	18	58	0.11026
3.0	2.275	0.131	21	49	0.13112
3.1	2.872	0.154	23	41	0.15436
3.2	3.593	0.180	26	35	0.17994
3.3	4.457	0.208	28	31	0.20773
3.4	5.480	0.238	30	27	0.23753
			31		

Table III (cont.).

Curve value or probit for expected kill	Expected percentage kill	Weighting coefficient	First differences	Relative no. of individuals for equal weights	$\frac{z^2}{pq}$
3.5	6.681	0.269	31	24	0.26907
3.6	8.076	0.302	33	21	0.30199
3.7	9.680	0.336	34	19	0.33589
3.8	11.507	0.370	34	17	0.37031
3.9	13.567	0.405	35	16	0.40474
4.0	15.866	0.439	34	15	0.43863
4.1	18.406	0.471	32	14	0.47144
4.2	21.186	0.503	32	13	0.50260
4.3	24.196	0.532	29	12	0.53159
4.4	27.425	0.558	26	11	0.55788
4.5	30.854	0.581	23	11	0.58099
4.6	34.458	0.601	20	11	0.60052
4.7	38.209	0.616	15	10	0.61609
4.8	42.074	0.627	11	10	0.62742
4.9	46.017	0.634	7	10	0.63431
5.0	50.000	0.637	3	10	0.63662
5.1	53.983	0.634	3	10	0.63431
5.2	57.926	0.627	7	10	0.62741
5.3	61.791	0.616	11	10	0.61609
5.4	65.542	0.601	15	11	0.60052
5.5	69.146	0.581	20	11	0.58099
5.6	72.575	0.558	23	11	0.55788
5.7	75.804	0.532	26	12	0.53159
5.8	78.814	0.503	29	13	0.50260
5.9	81.594	0.471	32	14	0.47144
6.0	84.134	0.439	32	15	0.43863
6.1	86.433	0.405	34	16	0.40474
6.2	88.493	0.370	35	17	0.37031
6.3	90.320	0.336	34	19	0.33589
6.4	91.924	0.302	34	21	0.30199
6.5	93.319	0.269	33	24	0.26907
6.6	94.520	0.238	31	27	0.23753
6.7	95.543	0.208	30	31	0.20773
6.8	96.407	0.180	28	35	0.17994
6.9	97.128	0.154	26	41	0.15436
7.0	97.725	0.131	23	49	0.13112
7.1	98.214	0.110	21	58	0.11026
7.2	98.610	0.092	18	69	0.09179
7.3	98.928	0.076	16	84	0.07563
7.4	99.180	0.062	14	103	0.06169
7.5	99.379	0.050	12	128	0.04979
7.6	99.534	0.0398	102	160	0.03977
7.7	99.653	0.0314	84	202	0.03143
7.8	99.744	0.0246	68	259	0.02459
7.9	99.813	0.0190	56	334	0.01903
8.0	99.865	0.0146	44	437	0.01457
8.1	99.903	0.0110	36	577	0.01104
8.2	99.931	0.0083	27	769	0.00828
8.3	99.952	0.0061	22	1037	0.00614
8.4	99.966	0.0045	16	1412	0.00451
8.5	99.977	0.00327	123	1947	0.00327
8.6	99.984	0.00235	92	2709	0.00235
8.7	99.989	0.00167	68	3812	0.00167
8.8	99.993	0.00118	49	5395	0.00118
8.9	99.995	0.00082	36	7764	0.00082

coefficient. The weighting coefficient will be sufficiently accurate if read only to the first two or three significant figures as given in column 3 of Table III, interpolating from first differences (column 4) if the provisional curve justifies an estimate to the nearest 0.01 probit. Each weighting coefficient then is multiplied (most conveniently on the slide rule) by the number, N , in the test to secure its correct weight, w , for calculating the dosage-mortality curve.

It has been specified, without further explanation, that the weighting coefficient is determined from the provisional regression line rather than directly from each separate observation. With this important exception, the weighting coefficient described above is equivalent to that proposed by Gaddum⁽⁵⁾ and by Hemmingsen⁽⁶⁾ for the same purpose. Gaddum has based his coefficients directly upon the separate p 's observed experimentally, so that above 50 per cent. kill the tests in which the mortality fell short of that expected would be weighted more heavily than those in which the mortality exceeded expectation. Conversely, below 50 per cent. kill, the excessive mortalities would carry greater weight than the deficient mortalities. Together these errors would bias the fitted regression line toward the horizontal. By using as a standard the probit (or mortality) determined from the experiment as a whole, instead of that shown by a single sample, the present weighting coefficients not only avoid this biasing error but give a suitable basis for comparing different dosage-mortality curves and for measuring their accuracy. Still another, though similar, weighting method has been used by McCallan and Wilcoxon⁽⁸⁾ in the reciprocal of their "error in concentration."

In planning an experiment so as to secure equally reliable results at all dosages and thereby avoid the necessity of weighting—with a corresponding simplification in the computations—more individuals should be used at high and low dosages than at intermediate ones. Equalisation will result if the experimenter treats with the dosage at each expected kill some multiple of the number of individuals listed in the fifth column of Table III. This shows that it takes three times as many animals to get the same accuracy at 95 per cent. kill as at 50 per cent. kill and nearly ten times as many at 99 per cent. as at 50 per cent. It would not justify the procedure followed in the experiments reported by Hemmingsen⁽⁶⁾, p. 40, in which nearly twice as many mice were used for the two middle of four concentrations of insulin as for the largest and smallest.

In order that each step may be clearly understood, a numerical example has been selected from Strand's⁽¹¹⁾ experiments with *Tribolium confusum*. Two of his series, designated as I and II, give the mortality of the adult flour beetle after five hours'

exposure to gaseous carbon disulphide, and these will serve to illustrate the various procedures of the present paper. There was no appreciable mortality in the controls, so that this factor did not need correction. The original data are given in the first four columns of Table IV. The next column, x , is secured from column 3 by reference to a table of common logarithms. With the exception of the probit values corresponding to 100 per cent. kill, the sixth column, y , gives the percentages in terms of the probits of Table I. The observed values for x and y were then plotted on cross-section paper (Fig. 3), and it is apparent from inspection that the two series, I and II, did not differ

Table IV.

Procedure for fitting the transformed dosage-mortality curve to kills of Tribolium confusum following 5-hour exposures to known concentrations of carbon disulphide. The computations in columns 7 to 10 and at the end of the table show the steps for fitting the regression line to the upper range of dosages from the data of both series (Fig. 3). Data from Strand (11).

Original data								$w x$	$w y$
Series no.	Total no. of insects	CS ₂ mg. per litre	% kill	x log. of dosage	y Probit kill	Weighting coefficient	w Weight	Column 8 \times Column 5	Column 8 \times Column 6
I	29	49.06	6.9	1.6907	3.517	—	—	—	—
	30	52.99	23.3	1.7242	4.271	—	—	—	—
	28	56.91	32.9	1.7552	4.557	0.555	15.5	27.20560	70.6335
	27	60.84	51.9	1.7842	5.048	0.633	17.1	30.50982	86.3208
	30	64.76	76.7	1.8113	5.729	0.500	15.0	27.16950	85.9350
	31	68.69	93.6	1.8369	6.522	0.292	9.1	16.71579	59.3502
	30	72.61	96.7	1.8610	6.838	0.125	3.8	7.07180	25.9844
	29	76.54	100.0	1.8839	7.952	0.0398	1.2	2.26068	9.5424
	30	49.06	13.3	1.6907	3.888	—	—	—	—
	30	52.99	20.0	1.7242	4.158	—	—	—	—
II	34	56.91	26.5	1.7552	4.372	0.555	18.9	33.17328	82.6308
	29	60.84	48.3	1.7842	4.957	0.633	18.4	32.82928	91.2088
	33	64.76	87.9	1.8113	6.170	0.500	16.5	29.88645	101.8050
	28	68.69	85.7	1.8369	6.067	0.292	8.2	15.06258	49.7494
	32	72.61	100.0	1.8610	7.447	0.125	4.0	7.44400	29.7880
	31	76.54	100.0	1.8839	7.952	0.0398	1.2	2.26068	9.5424
$S(w)$				$S(wy^2)$				$= 3931.653109$	
$S(wx)$				$S(wy^2) - \bar{y}S(wy)$				$= 103.156728$	
$\bar{x} = \frac{S(wx)}{S(w)}$				$\chi^2 = [S(wy^2) - \bar{y}S(wy)] - b[S(wxy) - \bar{x}S(wy)]$				$= 5.5564$	
$S(wy)$				$\{n' \text{ (see text)}$				$= 9$	
				$\{n = n' - 2$				$= 7$	
$\bar{y} = a = \frac{S(wy)}{S(w)}$				$V(a) = \frac{1}{S(w)}$				$= 0.007758$	
$S(wx^2)$				$V(b) = \frac{1}{A}$				$= 6.668327$	
$A = S(wx^2) - \bar{x}S(wx)$				$t \text{ (at } P=0.05)$				$= 2.365$	
$S(wxy)$									
$S(wxy) - \bar{x}S(wy)$									
$b = \frac{S(wxy) - \bar{x}S(wy)}{A}$									

consistently. In comparison with the remaining observations, the two lowest concentrations gave an exceptionally high kill. Over the remaining concentrations, the plotted values seemed to form a moderately straight line, so that the data were handled as two separate sets, only the results at 56.91 mg. of CS_2 per litre being included in both sets. The provisional regression lines were drawn in with the aid of a straight edge, but these provisional curves, indicated by the broken lines, agreed quite

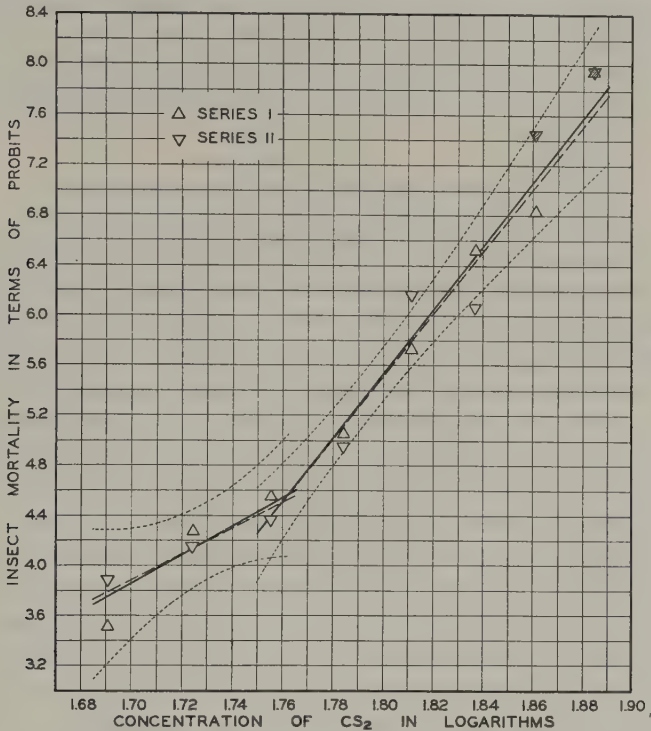


Fig. 3. A transformed dosage-mortality curve, showing the effect upon adult flour beetles of 5-hour exposures to different concentrations of gaseous carbon disulphide. The broken straight regression lines were placed graphically by inspection, the solid ones by computation, while the dotted curved lines show the limits within which the solid lines have been determined by the data. The shaded triangles represent treatments from which no beetles survived. Data from Strand⁽¹¹⁾.

closely with those arrived at by computation, the solid lines, in both the upper and the lower range of dosages.

Restricting our attention for the moment to the more important, upper range of dosages, the approximate curve was used first to secure probit values for 100 per cent. kills. We find that at a concentration of 72.61 mg. of CS_2 per litre, there was 1 survivor in Series I but 0 survivors in Series II, while no survivors were found in either series at 76.54 mg. per litre. The provisional curve showed that at a log. concentration of 1.8610 (72.61 mg.), 7.03 probits was expected and at a 1.8839 log. concentration

(76.54 mg.), 7.61 probits. Entering these values in column 1 of Table II, the two required probit values of 7.447 and 7.952 were obtained from column 3, using the first differences of column 4 for interpolation.

From the original plot of the provisional curve (on millimetre cross-section paper), the probit for each observed dosage could be read without difficulty to the nearest 0.01 probit. These were then entered directly in column 1 of Table III to secure the weighting coefficients from column 3 of the same table, interpolating with the aid of the adjoining column of first differences. The weighting coefficients so obtained were written down in column 7 of Table IV and multiplied on the slide rule by the corresponding number of insects (column 2) to determine the true weights in column 8. The last two columns of Table IV contain the products x multiplied by w , and y multiplied by w .

III. THE COMPUTATION OF THE REGRESSION LINE.

In toxicological experiments of the type which we have been considering, the mortality among a limited number of organisms is measured after treatment with known amounts of a toxic agent. These results have significance primarily because they form a sample from an infinitely larger group of organisms for which we are interested in determining the toxicological relationships. The fitting of a dosage-mortality curve is an attempt to infer from a given experiment the conditions obtaining in a class or species of organisms, and the calculated regression line of the dosage-probit diagram is the most accurate estimate which can be drawn from the data, granted that our basic assumptions are correct. In some cases it will be very near the first graphic approximation which has already been described, but oftentimes it will represent a rather important correction to this initial estimate, especially when the material is variable and fitting by eye less reliable. Moreover, in a calculated regression line, each separate observation can be weighted accurately, as has been shown, and the limits determined within which will lie the true curve for an infinitely larger population.

In describing the arithmetical procedure of fitting, the methods and symbols employed by Fisher⁽⁴⁾ have been adapted to the present purposes. Short-cut methods, suitable for use with a calculating machine, are described. With a machine, these should enable one to fit the regression line without previous experience.

The formula for the regression line may be expressed as

$$Y = a + b(X - \bar{x}), \quad \dots\dots(2)$$

where, in this case, Y is the mortality in probits on the regression line (or transformed dosage-mortality curve) which corresponds to any given dosage X , usually expressed in logarithms; $a = \bar{y}$ = numerically the average probit for all determinations in that part of the experiment which is being

fitted by a straight line; \bar{x} is the average of the dosages administered (in logarithms) for the same section of data; and b is the regression coefficient or the slope of the line, the amount by which the probit of mortality is increased for every unit increase in log. dosage. It is necessary, therefore, to calculate from the experimental data the quantities \bar{x} , \bar{y} , and b . The formulae are as follows:

$$\bar{x} = \frac{S(wx)}{S(w)}, \quad \text{.....(3)}$$

$$\bar{y} = \frac{S(wy)}{S(w)}, \quad \text{.....(4)}$$

$$b = \frac{S(wxy) - \bar{x}S(wy)}{A}, \quad \text{.....(5)}$$

$$A = S(wx^2) - \bar{x}S(wx), \quad \text{.....(6)}$$

where the symbols are defined as:

S = "the sum of" and indicates that all quantities of the type in the brackets after the S are to be added,

w = weight of a given observation, the product of the weighting coefficient multiplied by the number of killed plus survived,

x = a function of the dosage administered experimentally, usually its logarithm, and

y = the probit corresponding to the observed percentage mortality.

The position of the regression line, in the sense in which we will use the term, is determined by \bar{x} and \bar{y} , since it must pass through the point on the diagram given by these two means. They fix the degree of susceptibility to a toxic agent shown by the population as a whole. From a statistical viewpoint, b is the slope or the tangent of the angle with which the regression line will pass through the point established by \bar{x} and \bar{y} ; from a biological viewpoint, b measures how closely the individual organisms in the experiment agree with one another in their sensitivity to the toxic agent. It is convenient to express this toxicological characteristic as the percentage increase in dosage that is required to increase kill by one probit. This is the ratio of $100 \log_e 10$ to b , $\frac{230 \cdot 26}{b}$.

Returning to our numerical example, the solution of equations (3)–(6) has been given at the bottom of Table IV in the order which has been found the most convenient. The first, second, and fourth quantities are the totals of the last three columns of the table, while the two means were determined in order, without clearing the lower dials of the calculator, when the totals first appeared (in machines such as the Monroe and the Marchant). $S(wx^2)$ was obtained by placing wx on the keyboard of the calculator and multiplying by the corresponding x , then clearing the keyboard and upper dials

and repeating the process with the next pair of values until the total of the products, $S(wx^2)$, had been accumulated in the lower dials. Leaving this sum in the lower dials, $S(wx)$ was placed on the keyboard and subtracted \bar{x} times to secure A . Repeating the process with wx on the keyboard and multiplying this time by y , the sum, $S(wxy)$, was obtained directly. From $S(wxy)$ in the lower dials, $S(wy)$ on the keyboard was subtracted \bar{x} times to secure the next term, which, in turn, was divided by A to obtain the regression coefficient, b . In checking the arithmetic of these various operations, other short-cuts will soon suggest themselves for facilitating the work and reducing the possibility of error. It is important in this method that computations be carried out to six or more significant figures in the means and regression coefficient in order to insure sufficient accuracy throughout. From \bar{x} , \bar{y} , and b the equation of the corrected regression line was solved as $Y = 5.450 + 25.51 (X - 1.7967)$, holding for concentrations of carbon disulphide above approximately 57.8 mg. per litre of air. In this range, an increase in dosage of 9.03 per cent. $(230.26/25.5114)$ increased kill by 1 probit.

The change in slope at a kill of about 33 per cent. (Fig. 3) is a frequent phenomenon for which no explanation will be attempted here. A separate curve has been calculated for the lower concentrations, including the smallest dosage of the main curve. The regression coefficient, b , was less than one-half that for the higher dosages. Usually this lower section of the toxicity curve will be of too little practical or theoretical importance to warrant calculating its equation, and it may be questioned whether a straight line is the correct relationship when the mortality below 25 to 35 per cent. kill differs from the rectilinearity of the higher dosages. Assuming a straight line in the present case, the regression equation was $Y = 4.186 + 11.35 (X - 1.7286)$.

The two experimental series have been listed separately, although the same dosages were used in Series I and in Series II. If the number of living and dead for each dosage had been combined before calculating the percentage kill and transforming to probits, the regression equation would have been determined from half as many separate observations. The result should be practically the same. Tested arithmetically, the new equation, $Y = 5.436 + 25.33 (X - 1.7967)$, differed so slightly that both regression lines could not be shown in Fig. 3. When it is evident from the similarity of different experimental series that the stocks of test animals are the same, the results at each separate dosage may be combined into a single percentage and probit for placing the first regression line by eye and for reducing the labour of computing the curve, although for estimating the errors of this curve the longer form is preferred.

IV. ACCURACY OF THE REGRESSION LINE.

The fitting of a dosage-mortality curve to a series of experimental observations, however crude or refined the technique, is an attempt to infer, from a limited number of individuals, the "true" empirical relationship of dosage and mortality for a given toxic agent in an infinitely larger population from which they represent only a sample. The regression equation and line is the closest we can approximate this "true" relationship, but all determinations of this type are not equally reliable. If the experimental points are quite close to the line and the number of individuals is large, we have greater confidence that a second or third

determination will agree with our first estimate than if the points are scattered and based on fewer animals. We will want to compute from our experimental data not only the most likely position (the regression line) of the "true" dosage-mortality curve, but also how accurately this most likely position has been determined.

(1) *The χ^2 test for comparing observations with the computed curve.* The first step is to determine whether the observed mortalities agree with our original assumption of a rectilinear relationship on the logarithmic-probability scale within the limits of sampling error; in other words, do the experimental observations vary significantly from our fitted straight line? Since each observation has been weighted by the reciprocal of its variance (Nz^2/pq), which, in turn, is based upon a regression line at the observed dosage, the most satisfactory criterion is the chi-square (χ^2) test. At each dosage the observed mortality is compared with that expected from the regression equation, but instead of calculating separately each expected probit (mortality) from equation (2), and then subtracting it from the observed probit (mortality), a short-cut method for securing the sum of the squares of these differences may be adapted from the one given by Fisher (4). When this is combined with the weighting procedure above, which gives the part of the equation corresponding to the "expectation," χ^2 may be calculated quite easily as follows:

$$\chi^2 = [S(wy^2) - \bar{y}S(wy)] - b[S(wxy) - \bar{x}S(wy)]. \quad \text{.....(7)}$$

Nearly all of the components of equation (7) have already been computed in determining the regression equation. The first parenthesis contains $S(wy^2)$, which is the sum of the products of columns 6 and 10 in our example of Table IV. The second part is the numerator of the equation for the regression coefficient (equation (5)) multiplied by the regression coefficient, b . Although in this equation for χ^2 the weights, and therefore the expected probit values, are based upon the initial, graphic regression line, while the differences between expectation and observation depend upon the later, calculated regression line, the discrepancy thus introduced is not a serious one.

The computation of χ^2 is a relatively straightforward operation without statistical complications, but its significance depends upon a term known as the number of "degrees of freedom," n , which may be more difficult to evaluate. If the regression line were calculated from one set of data and then drawn on the same graph with the individually plotted points of a second, entirely independent series of determinations of toxicity, the second series could differ from the line in as many ways—

or in as many degrees of freedom (n)—as there are plotted points or observations (n'). Under these circumstances n would equal n' . If, however, the average log. dose and the average probit were calculated from the second series, and the regression line drawn through the point established by these two averages with a slope which had previously been computed from other data, the separate tests in the second series could not differ as freely from the line as before, because the position of the line has been determined from the observations with which it is being compared. The number of degrees of freedom would then be one less than the number of tests in the second series or $n = n' - 1$, for one degree of freedom has been used up in locating the position of the line. Finally, when not only the position of the regression line but also its slope have been computed from a given series of observations, the extent to which these latter may differ from the transformed dosage-mortality line is still more restricted. In this case, the one with which we have been dealing, the number of degrees of freedom would be equal to the number of separate tests less one which was sacrificed in using these same observations to determine the position of the regression line and less a second degree of freedom lost in establishing the slope of the line. The number of degrees of freedom in the regression line of our computations will be equal, therefore, to the number of separate tests in the series establishing the curve less 2, or $n = n' - 2$.

This rule is simple and easy to apply, but is complicated by another requirement, *i.e.* that the calculated distributions of χ^2 , upon which the tests of significance depend, are not very closely realised when very small numbers are expected. In fact, such tests are not rigidly exact when the number expected is less than 5. In toxicological experiments, the expected number of survivors at the higher dosages will regularly fall below this ideal limit, especially when zero survivors are obtained. If each of these particular tests is assigned a value of 1 in determining the number of degrees of freedom, the apparent goodness of fit will be exaggerated by the inclusion of observations which, because of their small weight, contribute little to the observed χ^2 . The exact procedure is to exclude from the computation both of χ^2 and of n the results of those dosages at which the number of *expected* survivors, based on the number of organisms counted and the regression line, is less than 3 to 5 individuals. An alternative, which is more convenient though possibly less precise, is to include these small contributions to χ^2 with their standard weights as before, but for the purpose of determining n' and n to group those in which the survival expectancy is small, so that there will be no contribu-

tions to n' or n which are based upon a survival expectancy of less than one individual. The limit of expectancy is lowered here because the separate observations will contribute somewhat more to χ^2 , despite their small weights, than they would if the variation between them could be smoothed out by combining them into as few terms as their contributions to n' . The same considerations would hold at the opposite end of the curve when the expectancy of death is very small.

Having secured χ^2 and n , it is a simple matter by reference to a table of χ^2 , such as Table III in Fisher's text, to determine if the observations depart more widely from our calculated dosage-mortality curve than could be expected by chance. If χ^2 is smaller than the value in the column for P equal to 0.05, the data may be considered consistent with the straight line that has been fitted. If the χ^2 is greater than the value corresponding to this probability (P), either the observations depart significantly from a straight-line relationship, or some uncontrolled condition in the experiment is causing a greater variation about the line than could be expected from simple fluctuations in sampling. Since systematic departures from rectilinearity were eliminated at the start, the second of these causes is more likely to be involved. Heterogeneity of this type does not necessarily invalidate the procedures described in the present paper.

(2) *The variances of position and slope.* The two parameters determined from an experimental series in calculating the regression line are those giving its position, a (or \bar{y}), and slope, b ; from the variance of a and of b we may determine how accurately they have been estimated. The square root of the variance of any statistical constant is its standard error, but since the variance must be computed in order to determine the standard error and is here much the more useful, we will deal with the variances directly rather than with their square roots, the standard errors. Since \bar{x} in the regression equation (2) is the independent variable, the average of the dosages selected by the experimenter for testing, it is not a "sample" from a "population" of dosages and is not subject to sampling error in the ordinary sense.

The regression line is calculated so as to intersect the point fixed by the average dosage and the average probit, so that the term a is numerically equal to \bar{y} , but since a is defined as a value on the regression line, its variance, $V(a)$, will be that about the regression line at a single dosage at or near the mean dosage, and hence considerably smaller than the variance of the observed probits for all dosages. The equation for the variance of a is

$$V(a) = s_a^2 = \frac{\chi^2}{nS(w)}, \quad \text{.....(8)}$$

where the symbols have the same significance as before.

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The variance of the regression coefficient, b , is given by the equation

$$V(b) = s_b^2 = \frac{\chi^2}{nA}. \quad \text{.....(9)}$$

The formulae for the variance of a and of b given in equations (8) and (9) represent the errors involved in the particular series of records from which they were calculated and are valid however great χ^2 may be. This comparison of χ^2 with its mean value n is a comparison of actual deviations with those theoretically to be expected from the numbers of units observed. If observation and computed curve agree satisfactorily within the limits of sampling error as tested by χ^2 (P greater than 0.1), the errors observed in such a specific experimental series may be replaced by a simpler form which will give the expected error for all similar tests involving the same dosages and numbers of organisms. The theoretical form for the sampling errors in a and b may be obtained from the fact that the mean value of χ^2/n is equal to 1. When the errors in a and b arise solely from the chance distribution of susceptibilities from one test to another, the calculation of their variances may be simplified to

$$V(a) = s_a^2 = \frac{1}{S(w)}, \quad \text{.....(10)}$$

and
$$V(b) = s_b^2 = \frac{1}{A}. \quad \text{.....(11)}$$

(3) *The zone of error of the regression line.* The best available estimate of the true dosage-mortality curve is the calculated regression line. The experience of statisticians indicates that if we can determine limits on either side of the regression line, such that there are 19 chances in 20 of their enclosing the true dosage-mortality curve, we will have a reasonable standard for prediction. Our next problem, therefore, is to determine the accuracy or "sensitivity" of the dosage-mortality curve which we have computed, using the margin of safety represented by 19 chances in 20 or $P=0.05$.

From the variance, $V(a)$, we can determine by how much the true regression line may lie above or below the most likely position as fixed by a , and from the variance, $V(b)$, we can find how much more or less it may be tilted. At the average dosage, \bar{x} , an error in b could have no influence upon the sensitivity with which a is an index to the true regression line, but as the dosage differs more or less widely from the average, both errors are of importance and will modify the accuracy of estimate of the true mortality corresponding to any given dosage. As

shown by Working and Hotelling⁽¹⁴⁾, the formula for the regression equation and its error may be written as

$$Y = a + b(X - \bar{x}) \pm t \sqrt{V(a) + (X - \bar{x})^2 V(b)}. \quad \dots\dots(12)$$

The value of t is not calculated but is taken from a table of "Student's" integral, such as Table IV of Fisher's text, from the column for $P=0.05$ at the value of n equal to the number of degrees of freedom for the curve. From equation (12) we may calculate the probit of kill and its error of estimate for a series of dosages covering the same range as our original experimental observations; from the plus errors draw a line above, and from the minus errors a line below the dosage-mortality curve such that there are 19 chances in 20 of these two boundaries, the branches of a hyperbola, enclosing the true dosage-mortality curve when transformed to the logarithmic-probit diagram. If it is preferred that the boundaries represent odds of 1 in 2, as in the familiar probable error, t is read from the column for $P=0.5$.

These different operations may now be illustrated from our example in Table IV, the computations for the main curve being summarised at the end of the table. For this range of higher dosages, $\chi^2=5.556$. Although the curve is based upon 12 separate determinations of mortality, the total number of survivors expected from the four tests at the two highest concentrations of carbon disulphide was only 1.36 beetles (1 survivor observed). Therefore these will count as 1 instead of as 4 in determining n' , and since $n=n'-2$, the number of degrees of freedom will be $9-2=7$. From a table of χ^2 , such as Table III in Fisher's text, the corresponding value of P lies between 0.5 and 0.7, so that the data may be considered consistent with the regression line which has been fitted to them. When the same test is applied to the line fitted to the range of smaller dosages, the χ^2 test again indicates satisfactory agreement

$$(\chi^2=1.404, n=4, P=0.84).$$

Since χ^2 indicates a satisfactory agreement between observation and fitted curve, the generalised form of the variances in the position and slope of the regression line may be used (equations (10) and (11)), when $V(a)=0.007758$ and $V(b)=6.6683$. We now have all the terms for computing the regression line and its errors (equation (12)) with the exception of t . For $n=7$, at odds represented by $P=0.05$, the value of t is given by Table IV in Fisher's text as 2.365. The equation for estimating the mortality in probits, Y , and its error within odds of 19 to 1, at any desired log. dosage, X , above a concentration of 57.8 mg. per litre, is

$$Y = 5.450 + 25.51(X - 1.7967) \pm 2.365 \sqrt{0.007758 + (X - 1.7967)^2 6.6683}.$$

The limits shown as curved dotted lines in Fig. 3 have been computed from this equation for the range of higher dosages and from a similar equation for the lower dosages. These boundaries define the accuracy with which the two solid regression lines have been determined by the experiment.

If the two series of tests had been combined, either when the experiments were made originally or in computing the percentages, that part of the error under the

square root would remain as it is in the longer form, since the generalised errors in position and slope depend only upon the sum of the weights and the variance of the log. dosage. The number of degrees of freedom would have dropped, however, from 7 to 3, so that t would have been increased from 2.365 to 3.182, and the limits of the estimated error increased proportionately.

V. APPENDIX. THE CASE OF ZERO SURVIVORS, BY R. A. FISHER.

The equations derived from the theory of large samples appropriate for plotting the points on the probit diagram, namely

$$q = \frac{s}{n}$$

and

$$\frac{1}{\sqrt{2\pi}} \int_x^\infty e^{-\frac{1}{2}t^2} dt = q,$$

give, for experiments with no survivors, $x = \infty$, with weight

$$\frac{z^2}{pq} \rightarrow zx \rightarrow 0.$$

It is evident that such values cannot, in this form, be used in fitting the regression line, and that the theory of large samples has broken down, as was to be expected, when the number in the class of survivors is small. A more exact treatment is necessary for such cases, and this is supplied by the Method of Maximum Likelihood.

If p is the probability of death, and q of survival, in any experiment, the probability that s survive out of n tested is

$$\frac{n!}{s!(n-s)!} p^{n-s} q^s. \quad \dots\dots(I)$$

In the method of maximum likelihood, we take the logarithm of the aggregate probability of all the experimental data, for any assigned series of probabilities of survival represented by the regression line, and estimate the position of the regression line by making this logarithm a maximum. This amounts to equating to zero the sum for the different experiments of the differential coefficients with respect to the value of x assigned. The exact form of the differential coefficient of (I) with respect to p is

$$\frac{n-s}{p} - \frac{s}{q} = \frac{qn-s}{pq}.$$

With respect to the probit value x , the differential coefficient involves also the factor dp/dx , and becomes

$$(qn-s) \frac{z}{pq}. \quad \dots\dots(II)$$

Now when both s and $n-s$ are so large that the distribution of s may be treated as normal, the factor $(qn-s)$, which is n times the difference

between the proportion of survivors expected and observed, is taken to be proportional to the difference between the probit values expected and observed, according to the formula

$$(qn-s) = n(x-X) \frac{dp}{dx} = n(x-X)z, \quad \dots\dots(III)$$

where X is the probit value expected, and x that observed. In such cases the equation for maximum likelihood is made up of such terms as

$$(x-X) \frac{nz^2}{pq},$$

and its solution consists merely in fitting the expected values, X , by least squares to observed values, x , obtained from each experiment, giving each observational point a weight nz^2/pq .

When, however, q is so small that s can frequently take values such as 0, 1, or 2, the equation (III) is not a satisfactory approximation, as is evident when $s=0$, for then x is infinite, while a finite value will be obtained from equation (III). If we write

$$n(x'-X)z = qn-s, \quad \dots\dots(IV)$$

then x' is a fictitious deviate, which, if assigned to any experiment with no survivors, will allow that experiment to exert its proper influence on the regression line. It will be observed that x' is a function not only of an observed frequency s/n , but also of X , the corresponding point on the regression line. It is only fictitious in the sense that it is not calculated from the result of just a single experiment, but requires also a knowledge of the expected value X inferred by fitting the regression line to other experiments. When $s=0$, $(x'-X)$ is always positive, so that the fictitious frequency to which x' corresponds is always less than that expected, as is evidently proper when the observed frequency is zero. The fictitious value x' , if used with its proper weight in recalculating a regression line of which an approximate estimate has already been made, will then allow experiments with few or no survivors to exert their proper influence in adjusting the line. It is of some importance to take this step, since the omission of experiments merely because they show no survivors must constantly bias our estimates in the sense of exaggerating the number of survivors to be expected.

When $s=0$, the value of x' depends only on X , though, of course, the weight assigned to the observation depends also on n , the whole number tested, equation (IV) becoming

$$x'-X = \frac{q}{z}.$$

These values are shown in Table II.

VI. SUMMARY.

The sigmoid dosage-mortality curve, secured so commonly in toxicity tests upon multicellular organisms, is interpreted as a cumulative normal frequency distribution of the variation among the individuals of a population in their susceptibility to a toxic agent, which susceptibility is inversely proportional to the logarithm of the dose applied. In support of this interpretation is the fact that when dosage is inferred from the observed mortality on the assumption that susceptibility is distributed normally, such inferred dosages, in terms of units called probits, give straight lines when plotted against the logarithm of their corresponding observed dosages. It is shown that this use of the logarithm of the dosage can be interpreted in terms either of the Weber-Fechner law or of the amount of poison fixed by the tissues of the organism. How this transformation to a straight regression line facilitates the precise estimation of the dosage-mortality relationship and its accuracy is considered in detail. Statistical methods are described for taking account of tests which result in 0 or 100 per cent. kill, for giving each determination a weight proportional to its reliability, for computing the position and slope of the transformed dosage-mortality curve, for measuring the goodness of fit of the regression line to the observations by the χ^2 test, and for calculating the error in position and in slope and their combined effect at any log. dosage. The terminology and procedures are consistent with those used by R. A. Fisher, who has contributed an appendix on the case of zero survivors. Except for a table of common logarithms, all the tables required to utilise the methods described are given either in the present paper or in Fisher's book. A numerical example selected from Strand's experiments upon *Tribolium confusum* with carbon disulphide has been worked out in detail.

It is a pleasure to record my indebtedness to Prof. R. A. Fisher, not only for the note appended to the paper, but also for invaluable advice throughout its preparation and for the facilities of the Galton Laboratory which have so generously been placed at my disposal. Among others who have been kind enough to read and criticise my manuscript, I wish especially to thank Prof. A. J. Clark, Dr F. Tattersfield, Dr J. O. Irwin, Dr A. B. P. Page, Mr H. H. S. Bovingdon, and Dr A. E. Brandt.

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PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

GENERAL MEETING of the Association held on Friday, November 9th, 1934, at 2.30 p.m. in the Botanical Lecture Theatre of the Imperial College of Science and Technology, London. The Chair was taken by the President, Dr T. GOODEY.

The following papers were read:

- I. The Economic Importance of Arachnids. By Prof. R. STEWART MACDOUGAL, M.A., D.Sc., LL.D.
- II. Applied Biology in the Control of the Worm Diseases of Domestic Animals. By E. L. TAYLOR, B.V.Sc., M.R.C.V.S., D.V.H.

I. THE ECONOMIC IMPORTANCE OF ARACHNIDS.

BY R. STEWART MACDOUGAL.

PROF. MACDOUGAL reviewed the subject with the aid of lantern slides, mentioning incidentally Scorpions and Spiders and dwelling more particularly on the Acarines or Mites, taking them family by family according as the families contained species of economic importance. The address was purposely given a veterinary bias, details being given of the proof that Louping or Trembling Ill—a disease of sheep in the Scottish Border counties and hill pastures elsewhere due to an ultra-microscopic virus—was conveyed from sheep to sheep by *Ixodes ricinus* as nymph and as adult. The researches at the Moredun Institute, Midlothian, had also established another disease of sheep known as Tick-Borne Fever.

II. APPLIED BIOLOGY IN THE CONTROL OF THE WORM DISEASES OF DOMESTIC ANIMALS.

BY E. L. TAYLOR.

(*Veterinary Laboratory, Ministry of Agriculture and Fisheries.*)

Parasitic worms are not essentially harmful.

PARASITIC worms are popularly regarded as among the most loathsome of all creatures; their "worminess" may be said to fill the average observer with a feeling of repugnance which leaves little room for tolerant consideration, and they are set down, one and all, as a cause of disease. There is, however, no essential connection between parasitic worms and disease, although in the popular mind there would appear to be so, and it is difficult to account for the prevailing uncritical outlook upon this point. There appears to be no natural tendency to regard the microscopic inhabitants of the intestinal tract as pathogenic; even such external parasites as fleas and lice, unless present in very

large numbers, are regarded rather in the light of an annoyance than a danger to health; but the detection of parasitic worms is at once looked on as a cause of head-shaking and irrefutable evidence of disease.

Nor is this outlook by any means confined to the man in the street, it is prevalent among scientific men in almost every branch of biology, and includes those who make a study of human and of veterinary medicine. One need not look far through the medical literature for ample evidence of this. At one time and another the cause of many diseases of unknown etiology has been ascribed to parasitic worms, merely because they both occurred in the same animal, and when unable to ascertain the cause of death a few parasitic worms found at post-mortem examination have often been regarded as the origin of a fatal disease.

How wrong an outlook this is can readily be appreciated by the parasitologist, or by any one who has made post-mortem examinations of healthy animals and observed the large number of parasitic worms which may be present. It is, in fact, the general rule to find some of these parasites in the lower animals, and the flock of sheep or the herd of cattle completely free from some of the very parasites which at times cause the most disastrous outbreaks of disease does not exist.

The margin of safety between normal and pathological infestation is a wide one.

The few observations which have been made indicate that the margin of safety between a harmless and a pathogenic infestation is a wide one. Whereas the average number of trichostrongyloid worms in healthy lambs is only a matter of hundreds, 15,000 or 30,000 are requisite for disease production, and affected ewes carry anything from 20,000 to 100,000; as many as 32,000 strongyloid worms have been found in a horse which had not shown any pronounced symptoms of disease and disease counts are probably very high. In an experiment recently carried out at Weybridge, in which chickens of a like age were infected with various numbers of a certain small tapeworm, *Davainea proglottina*, it was surprising to find that an infestation as high as 3900 worms did not produce any obvious signs of ill-health. Even the supposed baneful action of parasitic worms of the intestine, in introducing pathogenic bacteria into the body, has not been supported by experimental work, and, in general, the proper light in which to regard these creatures appears to be essentially as harmless, and, in many instances, as normal inhabitants of the body, only causing disease, as it were, by accident.

It is not my intention, however, to minimise the damage done by parasitic worms; evidence of the enormous losses which they continually occasion to farmers and keepers of animals of all kinds is to be seen on every hand. Last December a farm was visited in Surrey where, as a result of infestation with parasitic worms, only twenty-seven sheep were left alive out of 200, most of the twenty-seven being in a dying condition at the time of the visit. A careful enquiry on the Romney Marsh in Kent showed that between September 1st, 1933, and April 1st, 1934, forty-three of the farmers there lost 5131 of their lambs, representing over 15 per cent. of all the lambs born that year; and even when allowance is made for a 5 per cent. loss from other causes, the total of the estimated losses from deaths and poor condition amounts to some £10,300, or an average of £240 for each farmer. The colossal losses from liver fluke infestation which have occurred from time to time are well known. "Redworm

disease" is by far the most important disease of horses from the breeder's point of view. Very severe losses are also sustained from lungworms in cattle and sheep, and there are several other economically important diseases caused by parasitic worms in pigs, in poultry and in game birds; the rabbit keepers appear to have escaped rather lightly, but fox farming had hardly been recognised as a special industry before the lungworms began to make trouble, and now constitute the worst of the disease problems with which the fox farmer has to contend.

*The crowded conditions of animal husbandry have upset the
healthy host-parasite relationship.*

We have seen that parasitic worms are not essentially harmful, and it is obvious that any disease which they may cause is just as much a disease of the parasite as it is of the host, as it frequently leads to the death of both. Sheep perhaps suffer more severely from parasitic worms than do any other of the farm animals, yet it may be presumed that, in the primitive state, disease causation by parasitic worms was a comparatively rare accident: that primitive period might be expressed as a blissful state of "happy worms in happy sheep" in which all lived amicably together in perfect harmony where to live was to let live. But when man's intelligence began to rearrange, to make two blades of grass grow where one grew before, and to crowd five or ten or a hundred sheep or cattle where there had been only one, the environment for which the parasites had originally fitted themselves suffered a great change which altogether upset the mechanism controlling their numbers, and now, only too frequently, we see "unhappy worms in unhappy sheep," a state of disharmony and disease.

It is obvious that the origin of all our trouble is to be found in overcrowding. To produce larger and better crops and to keep more stock is, however, the whole aim of agriculture, and to recommend the keeping of less stock is lame advice to the farmer and savours of regression. It seems that the balance of host and parasite has been *permanently* deranged and, pending the time when we shall have better, cheaper and safer anthelmintics, we must make whatever compensations are possible, in this new order of things, to hinder the multiplication of the parasites.

*Where no suitable anthelmintic treatment is known control depends upon
hindering the process of the accumulation of worms in the host.*

Parasitic worms differ from bacteria, filterable viruses and protozoa in that they are unable to produce successive generations within the body of the host, and the production of disease by helminths is thus seen to be dependent upon the gradual collection of individual parasites by the host from its environment. Sanitary and other measures which only lead to a relative decrease in the intake of infective material, and which would be altogether inadequate for the prevention of its initial introduction, and so of no avail for the prevention of bacterial or virus diseases, may nevertheless be quite sufficient to maintain the numbers of parasitic worms below the figure requisite for disease production, and so be of practical value in the control of worm diseases. The disappearance of the parasitic worms of man from highly civilised countries is not due in any way to anthelmintic treatment, but to sanitation, and although animal communities can never compare with human communities in the perfection of sanitation, the small amount that can be done may, in the aggregate, be quite sufficient to prevent the appearance of disease.

The collection of infective material by the host may also be hindered by measures which do not strictly come under the heading of sanitation. Parasitic worms need to fit themselves more or less perfectly into two, three or four totally different environments, to a free life on the ground, and to a parasitic life in one, two or three different hosts. Each and all of these environments may be relatively favourable or unfavourable for the development of the parasite, and man may be able to do something to hinder their development and their collection by the host, and so prevent disease.

Where intermediate hosts are concerned, however, the range of possibilities for interference in their development is too great to cover in the small amount of time at our disposal, we should need to discuss such diverse topics as the control of fleas in dogs and the periodic appearance of swarms of dragonflies, so we must be content with one or two examples and I propose to confine my remarks to those forms which have a direct life history, and particularly to the strongyloid nematodes parasitic in grazing animals, which, from an economic point of view, form the most important group.

I do not wish to waste your time over an account of the ordinary hygienic measures practised on the farm, although there are one or two measures specially employed for the control of parasitic worms which I should like to run over with the aid of the lantern at the end of my paper. But I should like to consider one or two applications of a knowledge of the bionomics of parasitic worms to the control of disease, paying particular attention to the helminthic diseases of grazing animals.

*Dryness has a powerful effect in preventing the appearance of
most worm diseases.*

The parasitic worms which do not require an intermediate host all need to pass through a period of development of some days or weeks duration on the ground before they are ready to begin their parasitic existence.

In the early stages of their development on the ground the eggs and the pre-infective larvae are not particularly resistant to dryness or poisonous chemical substances, but the infective larvae are resistant to a very marked degree and are found to retain their vitality for more than twelve months. They are quite unable to withstand absolute desiccation, however, and the nearer the relative dryness approaches to that condition the sooner will they be killed. It is generally agreed that dryness is the most lethal of the natural forces acting on these resting infective stages, and usually correct to say that wet places are wormy places.

But it is not through its action upon the infective larvae that dryness takes its greatest effect in the prevention of disease, but upon the pre-infective stages, and the increase in the numbers of parasitic worms can be influenced to a marked degree by the condition of dryness under which animals are kept in an intensive system of rearing. Dry fox runs, dry pig pens and dry fowl houses are an efficient safeguard against parasitic disease, because the excrements of the animals have the opportunity of becoming dry before sufficient time has elapsed for the eggs or larvae of the parasitic worms to reach the more resistant infective stage.

Considerable benefit in the same direction may also be obtained by maintaining the grass of fowl runs very short through hard grazing with sheep. Excrement falling into long grass remains moist for days, the air which is entangled there being more or less saturated with moisture; but excrement falling on to short grass is exposed to the

ordinary atmosphere, to the drying action of air currents and of the sun, and has an opportunity of becoming dry before the larvae reach the less vulnerable infective stage.

The control of the *parasite* is only relative in these instances but the control of *disease* is absolute, the difference between tens or hundreds of eggs or larvae reaching the infective stages, on the one hand, and only units or tens on the other, may be quite sufficient to bring the disease under complete control.

In some instances dryness may encourage worm disease.

The effect of relative humidity upon the development of disease is, however, not always so easily understood, various ecological factors being concerned which may result in a helminthic disease being more prevalent under dry weather conditions than under wet conditions.

The partial knowledge acquired in the laboratory would lead one to suppose that wet seasons are the worst for the development of parasitic gastritis in ruminants or strongylidosis in equines. Field observations show, however, that the reverse is the case, and that some species of the parasites which cause this disease become much more abundant during or after prolonged periods of hot, dry weather than during the moister and cooler weather which is usually experienced in this country.

Dryness encourages some worm diseases in grazing animals.

(a) *Through increasing the intake of infective material.*

Three factors appear to be mainly concerned in the development of parasitic gastritis in sheep during periods of drought. Firstly the infective larvae of the worms which cause this disease are found to be attracted by a dim light but repelled by a strong one, and when the light is not too bright, as during the late evening, they ascend the blades of grass or other herbage of the pastures in the dew or rain on its surface, and descend again to the ground when the light becomes sufficiently bright to repel them in the early morning. After three or four weeks of this ascending and descending activity they show a tendency to settle down, and most of them come to rest near the ground. Quite a number are, however, stranded on the blades of grass when the dew evaporates, but even these tend to be near the ground as very few ever manage to ascend above the lowermost blade on a shoot of grass, the structure of the parts being such that they are turned off at the junction of leaf and sheath and prevented from proceeding up the stem; for every one found elsewhere ten or twenty may be found on the under side of the lowermost leaf.

It is obvious, therefore, that the greater part of the infection lies close to the ground, so that in a prolonged period of dry weather when the herbage becomes short and the animals are forced to bite nearer to the ground they may be said to acquire more infective larvae with every bite. But in addition to that, and possibly of greater importance, in order to satisfy their food requirements the animals need to graze more actively and for a longer period each day, so collecting more larvae from a wider area and being in danger of acquiring a pathogenic infection.

(b) *Through leading to an accumulation of infective material on the ground.*

The second factor concerned with the appearance of this disease during dry weather deals more particularly with the outbreaks of the disease *following* a period of drought, and concerns another point in the bionomics of the parasites. It has been found that some of the species of worms which are concerned in the production of parasitic

gastritis are extremely resistant to dryness while in the last stage of development within the egg, at the point at which the larva is just ready to hatch. At this stage they are even more resistant to dryness than at the infective larval stage, and have been observed to retain their vitality throughout fifteen months' air drying. Before they have reached that stage, however, and between the hatching of the egg and the development of the infective or third stage larvae they are relatively easily destroyed by dryness.

When the faeces of infected sheep fall on to the dry ground of a bare pasture, such as is commonly seen after a period of drought, they tend to dry very quickly, few, if any, larvae are able to reach the infective stage and some of the eggs are killed by dryness in the early stages of segmentation; the faeces often retain sufficient moisture, however, for many of the eggs to reach the resistant stage before dryness prevents all further development. There is thus the dangerous possibility of a gradual accumulation of *potential* infective material on the pasture during a prolonged period of drought, and if that period is followed by moist weather conditions of sufficient duration for the third-stage larvae to develop there may be a mass liberation of infective material and sudden widespread appearance of disease. If, on the other hand, a prolonged period of drought is broken by short rainy periods the potential infective material is destroyed, as the young larvae which come out of the eggs will be overtaken by dryness before they have time to reach the more resistant infective stage.

(c) *Through lowering the resistance of the host to the development of the parasite.*

The third factor at work in bringing about this disease during dry weather concerns the resistance of the host to the development of the parasites; as, however, there is not sufficient time to discuss even what little is known of immunity to parasitic worms it must suffice to say that the effect of deficient nutrition in lowering the natural or acquired resistance of an animal to parasitic worms has been clearly demonstrated in several instances. Sheep on a deficient diet have been found to acquire an infection more easily and to retain it for a much longer time than is usually done by sheep on a full diet. Extremely short pasture, and pasture which has been burnt by the sun, are known to be of a very poor quality, the quantity available to grazing sheep is also inadequate during times of drought, and there is no doubt that food deficiency is at such times of importance in producing worm disease.

The indications, therefore, are for a liberal allowance of concentrated foods during a prolonged period of drought, to keep up the resistance of the animals and prevent them from grazing too diligently, and for sheep which have been confined to certain pastures during a long dry period to be moved when wet weather sets in, and not to be returned to the dangerous pasture until the herbage has begun to grow again.

As has already been stated, it is the man-made environment of crowded animal husbandry that has provided the opportunity for parasitic worms to multiply to disease-producing numbers. If the weather conditions, the grazing conditions, the nutrition of the host, the state of the host's resistance and other factors influencing the development of the parasite are all in its favour, then disease is certain to result. If, however, these factors are against the parasite disease will not appear, and it seems quite possible that in some instances a very little difference in one direction or the other may swing the balance over to disease, or suffice to maintain it on the side of good health. It is the limitation of the multiplication of the parasitic worms by the

careful watching of all these points that the development of helminthic disease is prevented.

Some rule-of-thumb farming practices owe their beneficial results to limitations which they set to the increase of parasitic worms.

Some of the common farming practices which help to control parasitic diseases are as old as the hills and are carried out by rule-of-thumb, without any knowledge that the benefit which accrues depends upon their influence in limiting the multiplication of parasitic worms.

There is a system of folding sheep on arable land which completely controls the development of parasitic gastritis so long as the land is not already heavily infected when the sheep go on to it. This depends for its efficacy entirely upon moving the sheep forward on to clean ground and preventing them from wandering back on to ground which they have already cleared, more than four or five days previously; six or more days being requisite for the development of the infective larvae after the eggs have reached the ground.

The general tradition that land must "not be sheeped too heavily" and that sheep do better if they are frequently moved on to clean ground is very strong, and it is well known that sheep thrive badly if those rule-of-thumb precautions are not observed. In some instances the keenness of the farmer's observation is quite remarkable, the association between grazing on dewy grass in the early morning and the appearance of husk in young cattle being a good example. This tradition must have been very old before it was found that the larvae of the parasitic worms which cause the disease ascend the moist blades of grass in the dim light of the evening and descend again to the ground when the light becomes bright in the morning.

There are also certain interesting agricultural practices which have been built up in various localities for the control of worm disease. One of these, which is carried out on the Romney Marsh, might be mentioned in particular; it is practised by all the farmers there and during most years effectively prevents the appearance of parasitic gastritis in sheep. The pasture there is extraordinarily rich and is able to carry as many as seven or eight or even more sheep to the acre, a concentration which would certainly result in the appearance of parasitic gastritis unless something was done to prevent it. The lambs are born there about the 1st of April and kept on the pastures throughout the summer months; but about the 1st of September, after weaning, they are moved off the Marsh, and put out to graze on pastures in the surrounding counties, it usually being stipulated that the ground on to which they go shall have been free from sheep for not less than six months previously. The beneficial result of this practice may be explained as follows: The crowded conditions on the Marsh result in the infestation of the lambs being built up to a high degree throughout the summer months and sometimes the worms become sufficiently numerous before September 1st to cause disease, but as a rule the move to clean pasture takes place before this has occurred, the rapid rate of reinfestation is then suddenly stopped, the immunity of the lambs has a chance to operate, elimination of the many worms already present proceeds, and the danger of an outbreak of disease is averted.

Although intelligence without knowledge has been sufficient to enable the farmer to put several such hindrances in the way of the multiplication of parasitic worms, it does not appear to have carried him very far in some other directions. A tradition

against grazing sheep on successive crops on arable ground appears to be almost non-existent although this practice occasionally leads to very severe outbreaks of helminthic disease. It is not true, as recommended in some of the older books, that the infection may be finally disposed of by turning it in with the plough. Observations show that infective strongyloid larvae are able to make their way upwards through six inches of soil in as short a time as six or seven days, and in practice the ploughing and cultivation of the soil provides additional shelter and moisture for the larvae, and actually assists their longevity. In grazing on arable land a second time, therefore, after an interval of only six or twelve months, the sheep may be in serious danger of disease from a heavy infestation with these parasitic worms.

An intelligent application of the knowledge which we already have on the bionomics of parasitic worms should enable us to control disease production.

Finally it may be concluded that the application of laboratory research to the field is a branch of biological and medical work which too often is left to look after itself. We have seen that the limitation of the multiplication of parasitic worms suffices for the control of the disease which they are capable of producing, and that on account of their inability to give rise to successive generations within the same host this limitation of numbers is not too difficult to achieve, and there are some grounds for thinking that the accumulation of data which we already possess on the biology of parasites might be used more effectively for the control of parasitic disease.

REVIEWS

Field Studies in Ecology. By R. BRACHER. Pp. 100 with 10 text-figures.
J. W. Arrowsmith, Ltd. London. 1934. 2s. 6d. net.

A useful elementary introduction to the field ecology of plants "suitable for students in training colleges and universities, for field botanists and for senior scholars in schools." The book is simply and clearly written, with examples almost entirely from southern England, and students working through the course will gain an adequate knowledge of the identification and social relations of English flowering plants. Some of the references, books included in the bibliography and others not included are a little unexpected. Such theoretical aspects as there are in ecology are purposely omitted.

WILLIAM B. BRIERLEY.

Practical Bacteriology. By A. CUNNINGHAM. Pp. 203 with 26 figs. Oliver and Boyd. Edinburgh and London. 1934. 7s. 6d.

A useful practical book, which during the last ten years has proved its value as an introductory course for students of agriculture and of which a second edition has now appeared. The text has been largely rewritten and slightly extended, the greatest amount of new matter deriving from pH measurement and the bacteriology of milk and dairy products. Appendix 1 of the earlier edition which gave an outline classification of bacteria has been replaced by bacteriological tests for graded milk, and, throughout the book, new and more adequate methods have been substituted for older ones. The book frankly stresses the bacteriological viewpoint rather than the more modern and comprehensive microbiological approach, although lip service is paid to the latter in the use of the term "microbiological examination" of cheese, butter, soil, etc. Still even in dairy products the microfungi play a much larger part than students will realise from this course and in many aspects of the study of soil and farmyard manure they are almost if not equally as important as bacteria. Chapter vi on the bacteriology of plant diseases seems a little out of place in a book of this nature since this study is normally and more logically included in a course on plant pathology. Every reviewer of a practical handbook finds sins of omission and commission, his own favourite media, stains and methods not being included and others which he thinks of less value finding a place. The present book is no exception but as an introduction to agricultural bacteriology, which is all it claims to be, and not to agricultural microbiology which I think it ought to be this new edition is welcome.

WILLIAM B. BRIERLEY.

The Families of Flowering Plants. 2: Monocotyledons; arranged according to a New System based on their Probable Phylogeny. By J. HUTCHINSON. Pp. xiii + 243 with 107 figures. London: Macmillan and Co., Ltd., 1934. 20s. net.

Mr Hutchinson's first volume on the Dicotyledons appeared in 1926 and was immediately recognised as a major contribution to systematic botany. The present volume, on the Monocotyledons, attempts the same ideal as the earlier work, that is, the establishment of a phylogenetic system, and it follows a similar plan in arranging the families in a natural sequence, commencing with the most primitive types and ending with the most advanced. One is naturally and immediately confronted with

two fundamental questions; the monophyletic or polyphyletic origin of the Monocotyledons and the problem of what are primitive and advanced characters. Mr Hutchinson has had immense experience in systematic work and his studies have led him to conclude that the group is monophyletic in origin, that the herbaceous forms are more primitive than arborescent forms and that the Butomales and Alismatales, which share with the dicotyledonous Ranales the characters of an apocarpous gynoecium and numerous stamens, are the most ancient types. He also emphasises a character the value of which has not hitherto been generally recognised. This is the presence of a biseriate perianth which is regarded as being the basic feature of a whole line of descent. In his studies Mr Hutchinson has found it "necessary to put aside prejudices and ideas which have largely up to the present been accepted as botanical gospel" and his courageous attitude finds frequent expression throughout the volume. Perhaps the best example is his revolutionary view of the systematic value of the character of inferiority or superiority of the ovary. Regarding this as of less importance than the type of inflorescence, he has proposed new conceptions for the Amaryllidaceae and Liliaceae which result in a novel and probably more natural grouping of these large and crucial families. The author's ideas regarding the phylogenetic relationships of the Monocotyledons differ radically from those of Engler and they are shown in diagrammatic form on p. 7 and elaborated more fully in his tabulation of the families in the new system with notes on general tendencies which occupy pp. 9-16. Of course not everyone will accept Mr Hutchinson's views but their originality makes them very stimulating and they are worthy of the most careful consideration. It is very unfortunate that there are few or no fossil forms to which appeal might be made.

A key to the artificial groups of Monocotyledons leads to the main part of the book which contains straightforward and concise descriptions of the orders and families. There are also keys to the genera excepting those of the Orchidaceae and Gramineae. In these keys contrasting characters are shown by Clarendon Type lettering which effects a great saving of space but leaves one wondering what the author would have done in, for example, the Cyperaceae had there been one additional moiety in the hierarchy.

An interesting and valuable feature of the work are the illustrations which are line-drawings, of a useful size, many from the author's own pen of plants which, although not necessarily typical of each family, exhibit some point of special phylogenetic interest. The section on the Gramineae which is contributed by Mr C. E. Hubbard and is exceedingly well done is illustrated by excellent drawings of the floral details of all the tribes.

Reading through the volume one feels that masterly handling of data and sureness of touch which derives only from detailed knowledge and immense first-hand experience. A footnote to the Thismiaceae states "I have seen only a small proportion of these genera and cannot vouch for their distinctness" and that such a disclaimer should be needed once only in a volume covering so vast and comprehensive a field is remarkable.

In a rather delightful foreword, which deserves a place in botanical history for its aphorism "*Per Aspidistra ad Astra*," Sir Arthur Hill describes this volume as a "Genera Plantarum" in miniature for the Monocotyledons and this is undoubtedly the daily use to which it will be put by many a harassed teaching botanist. The book is appropriately dedicated to Agnes Arber and the author's exquisite surround to the dedication cleverly epitomises in decorative pictorial form his views of monocotyledonous phylogeny. The volume is well produced and misprints and slips are pleasantly rare.

The author's original, almost challenging treatment of his subject, and the novelty of his conceptions will naturally form matter for intensive consideration by technical systematists but there can be no doubt that his work and ideas are of quite first class importance. The present volume still further enhances the eminent position Mr Hutchinson made for himself by the publication of his first volume and Kew may well be proud of one whose work is in the great tradition.

WILLIAM B. BRIERLEY.

- (1) *Elements of Botany*. By R. M. HOLMAN and W. W. ROBBINS. 2nd edition rewritten and reset. Pp. 404 with 268 figs. Chapman and Hall, Ltd., London. 1933. 16s. 6d.
- (2) *A Textbook of General Botany for Colleges and Universities*. By R. M. HOLMAN and W. W. ROBBINS. 3rd edition. Pp. xiii + 626 with 463 figs. Chapman and Hall, Ltd., London. 1934. 25s.

New editions of two well known and popular textbooks characterised by clear unambiguous writing and quite unusually good illustrations. The first is an elementary introduction suitable for a one-term course and the second covers two terms' work. The authors' viewpoint is that a student "will profit by having the subject related wherever possible to agricultural practices and problems, and by the use of economic plants for illustrative material in every case where they will serve the particular end as well as any other plants." This viewpoint is explicit in the chapter on the fungi but is not very apparent in other chapters. Both books have been revised throughout and slightly enlarged as compared with the previous editions but the general organisation and treatment remain the same. In the larger work three pages on the climax in plant succession have been added from the pen of F. E. Clements and six pages on fossil plants by R. W. Chaney have been added to the chapter on evolution and heredity. There is also a useful and well-selected list of books in English for reference and collateral reading. As compared with the second edition there are about fifty additional illustrations and a number of the older figures have been replaced by better pictures.

Many American textbooks are too exclusively American in outlook, textual matters, examples and references to find any place in other countries but this College text does not suffer in any marked degree from such scientific nationalism. It is an extremely good introduction to the study of plants and the authors' sympathy with the applied outlook makes it unusual among botanical textbooks. Both volumes are beautifully printed and bound and contain unusually good indexes.

WILLIAM B. BRIERLEY.

Texas Grasses. By W. A. SILVEUS. Pp. xlvii + 782. The Author, 832 Cambridge Oval, San Antonio, Texas, U.S.A. 1933. \$7.50.

During the last fifteen years, owing primarily to the leadership of A. S. Hitchcock, Agrostologist to the Department of Agriculture of the United States, the grasses have received a very large amount of attention in America and, in general, they are, perhaps, the most thoroughly explored moiety of the American flora. This is not surprising in a country depending so largely upon agriculture and the value of these researches is to be measured not only by the enlargement of scientific knowledge but by the immense practical applications which have ensued. For those botanists, however, who are not specialised agrostologists, the grasses still remain a difficult group of plants, easy to recognise but hard to identify, and this position is realised most clearly when, as so often happens in agricultural botany, one is confronted with specimens from an alien flora. Any larger work dealing soundly with systematic agrostology is, therefore, particularly welcome.

Texas, with its great area and varied range of habitats and climatic conditions, shows a corresponding diversity in its grasses. The state possesses about 550 species of grasses, representing 13 of the 14 tribes and nearly all the genera of the United States. Further, in the United States there are about 1100 to 1200 species, which is about one-fifth of the total number known to science. A book on Texas Grasses is therefore of very considerable importance to all students of this group of plants and it may be said at once that the present volume is a noteworthy contribution to descriptive systematic agrostology.

Following a brief and well-illustrated general introduction there is a useful and rather extensive glossary of the special terms used in the description of grasses. After a few practical hints on how to identify grasses the author passes to descriptions of the sub-families with a key to the tribes, and descriptions of the tribes with keys to the genera. All this, which occupies some 34 pages, is introductory to the technical portion of the book, some 770 pages, which contains descriptions of the genera and species arranged under the following tribes: 1, Bambuseae (Bamboo tribe); 2, Festuceae (Fescue tribe); 3, Hordeae (Barley tribe); 4, Aveneae (Oat tribe); 5, Agrostideae (Timothy tribe); 6, Zoysieae (Curly Mesquite tribe); 7, Chlorideae (Gramma tribe); 8, Phalarideae (Canary-grass tribe); 9, Oryzeae (Rice tribe); 10, Zizanieae (Indian-rice tribe); 11, Paniceae (Millet tribe); 12, Andropogoneae (Sorghum tribe); 13, Tripsaceae (Corn tribe).

In dealing with each genus the distinguishing characters are first given, then brief general notes on the ecology, distribution, economic value and botanical characters of the species, a key to the species and, finally, the detailed diagnosis of the individual species with notes on their occurrence in Texas. Each genus is followed by a series of illustrations of the general habit of all the species with clear line-drawings of the dissected spikelets and flower characters. There are 420 full-page figures of which 320 are photographic reproductions and the remainder line-drawings and these illustrations are a striking and valuable feature of the book.

In his treatment of the sub-families, tribes and genera the author has followed the same sequence of arrangement as in Hitchcock's classical *Genera of Grasses of the United States*, and the descriptions of the family, sub-families, tribes and genera, as well as the keys, have been adapted from this work. The nomenclature throughout is that of the United States Department of Agrostology. In fact the present volume is largely the application of Hitchcock to the grasses of Texas and it is a remarkably successful application. The use of different types in the keys and species diagnosis make these very easy to follow, and the use of diacritical marks in the glossary, and for botanical names in the text, is an interesting and novel feature which should have a valuable tendency in standardising pronunciation. The book closes with a bibliography of twenty-three American works on systematic agrostology and a good index. The text is remarkably free from misprints and the volume is most attractively produced.

The author is apparently not a professional botanist but a lawyer for whom botany is a hobby. One can only say that rarely in the history of botany has an amateur produced a volume of such professional thoroughness and scientific merit. The book is a splendid piece of work and the author is to be congratulated on his achievement.

WILLIAM B. BRIERLEY.

Economic Plants. By E. E. STANFORD. Pp. xxiii + 571 with 376 illustrations. D. Appleton-Century Co., N.Y. and London. 1934. 21s. net.

The author's viewpoint is stated clearly in his preface. It is that "the utilities of plants, as well as their life and activities, have their proper claim upon a place in the educational program" and that "economic botany may occupy a very significant position in the teacher-training curriculum." The book is designed to "formulate a brief survey of several of the more important groups of plants and plant products utilized by the human race. While it is hoped that it may be useful to the professionally inclined student of plant science, it is planned primarily for that much larger group of students who wish to extend their knowledge of the kingdom of living things about them, the living green things upon which, in the last analysis, all human life depends."

The first two chapters are an elementary introduction to the classification and morphology of plants in general. The next four chapters deal with forests and forest products such as wood, timber and lumber, resins and related products, tanning materials and cork, latex and rubber. Chapter VII is devoted to textile plants and

the various fibres derived from them and Chapter VIII to paper and pulp. In Chapter IX grain and forage crops are described and in Chapter X sugar and sugar plants. Chapter XI is given to fixed oil plants and their products. Chapter XII opens with a brief discussion of the nitrogen relationships of plants and then describes leguminous crops and nut plants. Grapes, citrus and various Rosaceous fruits are dealt with in Chapter XIII and spices, volatile oils and flavourings in Chapter XIV. Chapter XV is devoted to beverage yielding plants and Chapter XVI to medicinal plants.

No attempt is made to follow a hard and fast mode of presentation but in discussing the various plants the author usually begins with the origin of the plant and its history in cultivation, passes to a description of the plant and its culture and then gives an account of the commercial importance and uses of the products and the manufacturing processes involved. As the volume covers such an enormous field the accounts of individual plants and their products are necessarily brief being merely sufficient to whet one's appetite. Unfortunately practically no references are given or indications of any sources where one might obtain more detailed information, and there are no suggestions for further reading. These are serious omissions from the volume since many of the author's descriptions are not entirely adequate and keen students will certainly ask for more.

The book is written by an American essentially for Americans and largely neglects such plants and their products as do not figure in American usage or "occidental trade." The author states that in his book "a number of important plant groups such as the edible 'vegetables' and the ornamental plants are conspicuous by their absence" and that "alcoholic beverages, for various reasons, are beyond the purview of this text." Even allowing for this, however, there are many plants and products omitted which might well have found a place. Thus, whilst malting barley is included, the hop plant is not mentioned: *Phoenix sylvestris* is noted as the source of Jaggery but *Phoenix dactylifera* which gives the much more important edible date is passed over; numerous American woods are described but teak is omitted, and so forth. Some of the omissions are rather serious and tend to reduce the value of the work.

The information given seems to be accurate and up to date although the author does not seem to be well acquainted with recent Russian work on the origin of cultivated plants. The writing is, on the whole, clear and unambiguous, although here and there occur sentences which make one blink, e.g. "Ginger scattered with developing trade"; "The nativity of the spice was later discovered by the Portuguese and more extensively exploited by the Dutch"; "The Oriental conscience (is) most untrustworthy"; "Battling for spices, Portuguese, Dutch, French, and English left a trail of blood in the Indies and in India. Spices to-day are a minor issue there, but the end of the trail of blood is not yet"; "The prohibition of alcoholics resulted in a lessening of barley acreage in the United States"; "In the summer of 1931, wheat sold for what was then an all-time low in Liverpool"; "That which dies and decays is not 'wasted,' but, as humus, remains, perhaps, for some future use which the anthropocentric mind may conceive as 'utility'"; "Castor oil is somewhat employed in transparent soaps"; "Coffee has migrated experimentally all over the agricultural tropics. It came to the West Indies in 1720." Also facetious comments are really out of place in a serious volume on economic plants.

The book contains a rather detailed table of contents, which is a useful feature, and a not very adequate index. There are 376 good illustrations, a few of which are original. The book is a little disappointing because it might easily have been so very much better. It is, nevertheless, a very useful production and as it contains a great amount of information marshalled in orderly sequence, data which are otherwise often rather inaccessible, it is a volume that many of us will be glad to have on our own shelves for easy reference and one that should find a place in botanical libraries. Considering the format of the book the price is not excessive.

WILLIAM B. BRIERLEY.

The Wistar Institute Style Brief. Prepared by the co-operative efforts of the editors of journals published by the Wistar Institute and the Staff of the Wistar Institute Press. Pp. 170, with 23 text-figures, and 37 plates. The Wistar Institute of Anatomy and Biology, 36th Street and Woodland Avenue, Philadelphia, Pa., U.S.A. 1934. Price \$ 2.00.

From time to time every editor of a scientific journal has a little difficulty with some contributor since it is a rare manuscript indeed that does not need editorial attention. And, after some particularly exasperating case, probably every editor has toyed with the idea of producing a booklet containing detailed advice to authors on how to write, prepare and illustrate research work for publication. Speaking from my own experience as hon. editor of the *Annals* since 1921 I have found that my editorial privileges include not merely the reading and emendation of manuscripts and the giving of fatherly advice to their authors, not merely the correcting of authors' proofs or the correcting of the authors' corrections, but often the recasting of more or less extensive portions of manuscripts and tables, the replanning or even redrawing of figures and graphs and, in two cases, the entire rewriting of papers. This, of course, is all part of the game and thanks are not expected and rarely received. It would however greatly simplify matters, reduce editorial work and publication expenses, and make the papers themselves better and more efficient to their purpose, if scientific workers would give more thought to the preparation of their manuscripts and illustrations and take the trouble of finding out the best way of achieving their end which is early and adequate publication of their results.

The ten scientific journals issued by the Wistar Institute of Philadelphia are widely known, and as widely appreciated, not only for the excellence of their contents but for their efficient format. Their hon. editors, however, seem to have passed through much the same experience as I have with the *Annals* and, in consequence, have combined with others to produce *The Wistar Institute Style Brief*, which is a guide for authors in preparing manuscripts and drawings for the most effective and economic method of publishing biological research.

In the first half of the *Style Brief* are given simple plain instructions for the preparation of manuscripts and illustrations with numerous examples of right and wrong methods. The second half contains thirty-seven plates from Wistar Institute journals showing different methods of black and white and colour reproduction, right and wrong methods of lettering, scale reduction, etc. Some of these illustrations, especially Plate 6, which is reproduced by four-colour process, are very beautiful. The *Style Brief* is an exceedingly efficient production and could be read usefully by all intending contributors to scientific journals.

WILLIAM B. BRIERLEY.

Cellular Respiration. By NORMAN U. MELDRUM. Pp. xi+116 with 17 figures. Methuen and Co., Ltd. 1934. 3s. 6d. net.

Respiration is the organised oxidation of organic metabolites, whereby work may be derived and turned to the use of the cell, and the aim of this book is to describe the mechanisms by which the molecules oxidised are burned in the cell. The mechanisms which enter into respiration of brain, muscle, yeast and other living cells are dehydrogenase systems; the "Atmungsferment" of Warburg, oxidases and peroxidases, Keilin's cytochrome and, finally, glutathione. The book is a rather theoretical biochemical essay on these mechanisms. In an interesting introductory chapter the author is at some pains to point out how little we know of the more fundamental issues involved, e.g. "It is as yet impossible to say how a molecule breaks down to carbon dioxide and water"; "At the present moment it is impossible to define an enzyme except by the way in which it acts"; or "It is, for example impossible to describe the

respiration of nervous tissue; the fact is that almost literally nothing is directly known on the subject." In the last chapter on "Modern Developments" the author considers developments of technique in enzyme chemistry and the views they have led to. In brief the modern study of respiration has led (1) to the emphasis of molecular specificity and the dominance of organic structure in determining catalytic properties; (2) to the discovery of the widespread occurrence of haematin catalysis; and (3) to the probability that the sensitivity of respiration to cyanide and sulphide is due to the latter combining with haematin catalysts.

There are references at the end of each chapter, a glossary, a short appendix describing the use of the Thunberg tube and the Barcroft differential manometer, and a good index.

WILLIAM B. BRIERLEY.

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THE INFLUENCE OF SEASON AND OF THE
APPLICATION OF LIME ON THE BOTANICAL
COMPOSITION OF GRASSLAND HERBAGE

BY WINIFRED E. BRENCHELEY, D.Sc.

(From the Botanical Department, Rothamsted Experimental
Station, Harpenden, Herts.)

(With 5 Text-figures.)

ANNALS OF APPLIED BIOLOGY
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yield and botanical composition of hay. An area of long-established grassland was divided into strips dressed with various combinations of manures, chiefly artificial, and with certain modifications the same treatment has been continued annually till the present day. In 1903 a system of periodic liming every four years was instituted on most of the plots, and in 1920 the remainder were brought into line. During the period 1856-1919 the changes in the herbage were recorded by frequent analyses of hay samples into the three main groups of grasses, leguminous and miscellaneous plants, while on seven occasions complete separations into individual species were made and the results published in detail (1, 2). By 1919 consideration of all the results focused attention on two further problems:

(1) Given constant treatment, how far is the botanical composition of the herbage influenced by seasonal variations from year to year?

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(2) How quickly is the botanical composition of grass cut for hay affected by change of manurial treatment, apart from seasonal variation?

In 1920, various plots were selected for complete botanical analysis over a period of years.¹ These included three representative plots of which the unlimed halves had had no change of treatment since 1856, and had received no manure, mineral fertilisers only, and minerals and sulphate of ammonia respectively, together with all those plots which first came into the liming system in 1920. Certain other analyses were also made for special purposes dealt with later. This provided material from a group of plots in which the herbage might be considered to have reached a position of equilibrium as far as direct manurial influence is concerned, and from another group in which the botanical composition passed through a stage of flux owing to the change of treatment. In some plots of the second group the complete analyses were only carried out in alternate years, while in the first they were made annually for at least five years. Partial separations into groups have been made on the same plots where the time factor precluded further complete analyses, and these have been continued until the present day.

The history of the plots considered is here tabulated for reference to avoid undue repetition later.

GROUP 1. *Plots with continuous manurial history.*

Sampled yearly.

Plot 3, U. and L.	Unmanured.
Plot 7, U. and L.	Complete mineral manure: super; sulphates of potash, soda, and magnesia.
Plot 9, U. and L.	Complete mineral manure and sulphate of ammonia (=86 lb. N).

GROUP 2. *Plots with change in manurial treatment and/or later liming.*

Sampled yearly.

Plot 14, U. and L.	Complete mineral manure and nitrate of soda (=86 lb. N).
Plot 18, U., L.L. and H.L.	Mineral manure (without super), and sulphate of ammonia (=86 lb. N), 1905 and since, following minerals and ammonium salts supplying the constituents of 1 ton hay, 1865-1904.
Plot 19, U., L.L. and H.L.	Farmyard dung in 1905 and every fourth year since (omitted in 1917), following nitrate of soda (=43 lb. N) and minerals, 1872-1904.
Plot 20, U., L.L. and H.L.	Farmyard dung in 1905 and every fourth year since (omitted in 1917); each intervening year plot 20 receives sulphate of potash, superphosphate and nitrate of soda (=26 lb. N), following nitrate of potash and superphosphate, 1872-1904.

¹ It is hoped to publish the complete analytical data in the *Report* for 1934 of the Rothamsted Experimental Station. In the present paper skeleton tables only are given of a few species to illustrate salient points.

Sampled in alternate years.

Plot 5 ¹ , U.	Unmanured, following ammonium salts (=86 lb. N), 1856-97.
Plot 5 ² , U.	Superphosphate, and sulphate of potash, following ammonium salts (=86 lb. N), 1856-97.
Plot 15, U. and L.	Complete minerals as plot 7, following nitrate of soda (=86 lb. N), 1858-75.
Plot 17, U. and L.	Nitrate of soda (=43 lb. N).

U.=unlimed; L.=limed 1920 and every four years after;
L.L.=light limed; H.L.=heavy limed.

A. CHANGES IN HERBAGE DUE TO SEASON.

The influence of season on the constitution of the herbage is reflected in the relative proportions of the main groups of grasses, leguminous and miscellaneous species, and in the variation in the proportions of the individual species within these groups.

The analytical data available is somewhat unwieldy, and for the sake of simplicity attention will be concentrated on a group of plots giving a representative range of manuring, *i.e.* no manure, and mineral fertilisers with and without nitrogen applied as sulphate of ammonia and nitrate of soda respectively (plots 3, 7, 9 and 14). In the first three cases the limed areas can also be considered, as the influence of lime had become stabilised since its first application in 1903. Pertinent instances from other appropriate plots will be cited where applicable.

It is impossible to give the fifteen years' meteorological data in full, but some idea of the range of seasonal variation may be gained from Table I, which gives the essential figures on a three-months basis.

Table I.
Meteorological data, 1919-33.

	Rainfall (total) in.				Bright sunshine (total) hours				Temperature (mean) ° F.			
	Jan. to Mar.	Apr. to June	July to Sept.	Oct. to Dec.	Jan. to Mar.	Apr. to June	July to Sept.	Oct. to Dec.	Jan. to Mar.	Apr. to June	July to Sept.	Oct. to Dec.
1919	11.3	5.4	7.5	8.9	188	609	507	218	35.9	52.0	57.5	40.5
1920	5.2	7.8	8.3	5.9	277	565	409	254	41.9	52.3	56.2	43.5
1921	3.7	3.2	4.0	5.1	253	641	559	270	42.7	52.1	61.4	45.3
1922	7.9	6.1	10.4	5.3	272	659	379	252	39.1	51.3	55.4	42.7
1923	7.9	3.8	8.7	9.6	189	398	670	245	41.5	49.4	59.4	41.1
1924	4.8	9.8	10.5	11.5	287	548	524	158	38.0	51.9	57.8	45.4
1925	7.2	4.3	10.7	7.4	210	604	441	251	40.0	51.8	57.9	41.8
1926	6.2	7.9	5.8	8.5	206	443	480	208	41.8	51.2	60.3	42.3
1927	8.8	6.6	12.9	8.2	234	576	420	184	40.5	50.9	57.8	41.8
1928	8.6	4.6	5.5	9.8	258	527	681	248	41.1	50.5	58.9	43.9
1929	2.6	5.7	2.3	17.1	291	643	646	273	35.6	50.2	61.2	44.5
1930	5.6	6.2	8.5	9.2	231	524	546	243	39.6	52.1	58.7	43.7
1931	4.5	7.5	9.5	5.0	284	486	434	228	38.0	51.7	56.4	44.2
1932	4.4	7.5	6.7	7.3	262	485	441	208	39.0	50.3	60.0	43.7
1933	7.1	3.5	4.6	3.0	370	562	673	187	39.4	53.3	62.7	41.3

(1) *Seasonal variation in the proportion of grasses, leguminous and miscellaneous species.*

With complete dressings of minerals and nitrogen (plots 9 and 14) the proportion of grasses is very high, sometimes reaching 99 or 100 per cent., and as a general rule is little influenced by season. Occasionally the grasses are somewhat depressed by an abnormal development of some miscellaneous species, as *Rumex acetosa* on plot 9 in 1919, and *Anthriscus sylvestris* on plot 14 in 1924 and 1925. In no instance has the almost negligible proportion of leguminous plants been significantly increased.

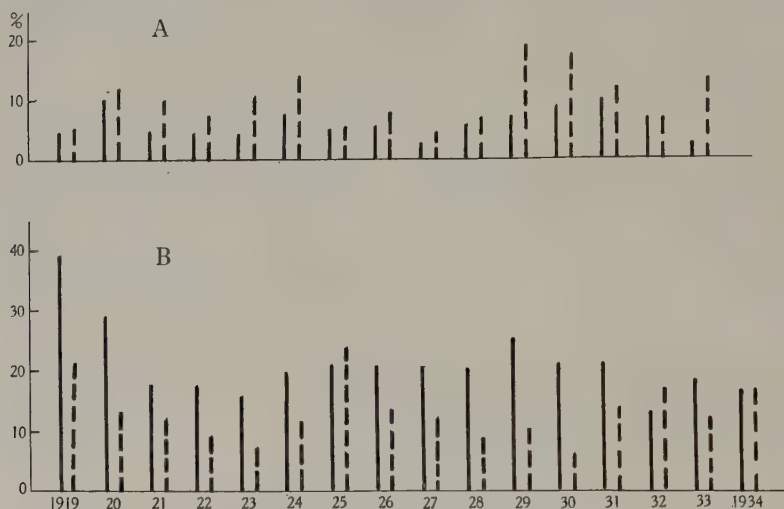


Fig. 1. A. Percentage of leguminous plants on plot 3 (unmanured), 1919-33. B. Percentage of miscellaneous species on plot 7 (minerals), 1919-34. ——— Unlimed. - - - - Limed.

The omission of phosphate from an otherwise complete fertiliser (plot 18) has prevented the grasses from becoming so entirely predominant. *Rumex acetosa* has maintained its position, and in some seasons, as 1922, 1926 and 1930, has constituted about 20 per cent. of the herbage. This affords an interesting quantitative corroboration of various observations hitherto made that *Rumex acetosa* is more encouraged by deficiency of phosphate than by acidity of soil.

On unmanured plots and in the absence of nitrogen wide seasonal fluctuations occur. Without either manure or lime (plots 3 and 5¹) an increase in grasses is usually accompanied by a decrease in miscellaneous plants, the variation in leguminous species being comparatively small.

With minerals the grasses and Leguminosae vary in correlation, the miscellaneous plants remaining much more constant. Where, however, minerals have followed several years of nitrogenous manuring (plots 5² and 15) the seasonal variations are distributed between all three groups. Liming tends to exaggerate the range of variation, upsetting the relative constancy of the leguminous plants on the unmanured and the miscellaneous species on mineral plots (Fig. 1).

The use of farmyard manure with or without artificials seems to introduce a certain rhythm into the seasonal fluctuations, which tend

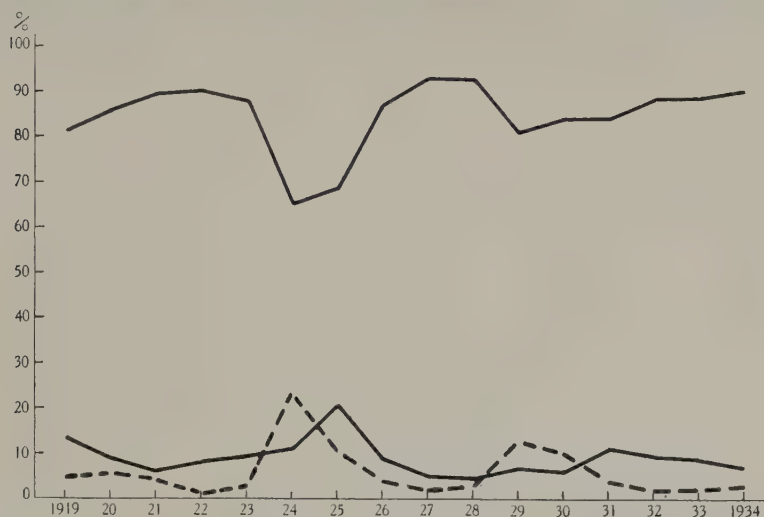


Fig. 2. Curve showing rhythmic change of proportion of the three main groups of species on plot 20, unlimed (dung and artificials), 1919-34. — (upper) grasses. — (lower) miscellaneous. - - - - Leguminous species.

to increase to a maximum and then decrease to a minimum over a period of years (Fig. 2). Three of these rhythmic cycles can be traced between 1919 and 1934, the peaks of maxima and minima not always occurring in the same year in the three groups of species.

The direction of change in the proportion of the three groups year by year is not always the same with different treatments; nevertheless, in some years characterised by outstanding meteorological conditions most plots behave very similarly. For instance, in 1921 the proportion of grasses was high on most plots in a season of low rainfall, medium sunshine and comparatively high temperatures during the growing season. In 1924 the same plots gave a low percentage of grass with a

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medium amount of rain, low temperatures early in the season, and much sunshine in March and April. Similar conditions gave similar results in 1929 on the unmanured and mineral plots (Fig. 3). It would seem that low temperatures early in the season tend to reduce the proportion of grasses, even though abundant sunshine occurs about April and May. On the other hand, relatively high temperatures early in the year encourage the grasses even with low rainfall and average amounts of sunshine.

The question arises as to whether the annual variations in yield have any direct influence on the botanical composition of the herbage. Comparison of the figures over a varied range of manurial treatments fails

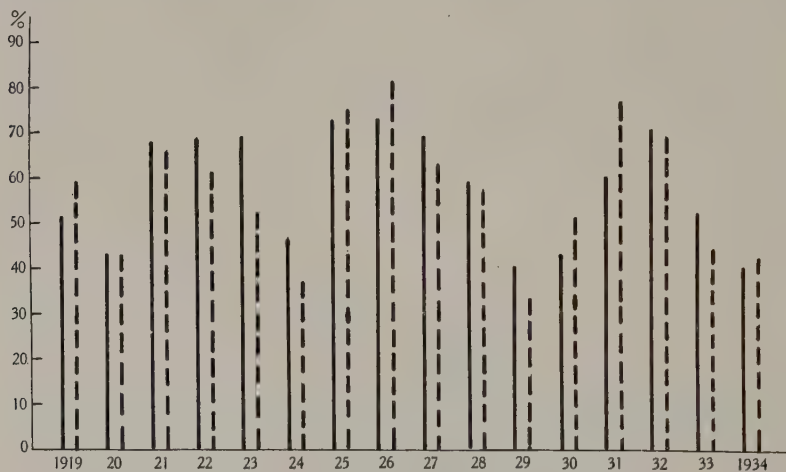


Fig. 3. Percentage of grasses on plot 7 (minerals), 1919-34.

—— Unlimed. - - - - Limed.

to reveal any correlation between the yields and relative proportions of grasses and miscellaneous plants. Though no definite association can be traced, with nitrate of soda and minerals (plot 14) the only two serious drops in the proportion of grasses and corresponding increases in miscellaneous species occurred in years of high yield; but this may be merely coincidence. With mineral manures, however, there is some suggestion of association between heavy yield and high percentage of leguminous plants, particularly on the limed areas. In 1920, 1924, 1929 and 1930 the proportion of *Lathyrus pratensis* was particularly high, ranging from 45 to 57 per cent. with lime and from 28 to 35 per cent. unlimed. In all cases except one (1929, plot 7 unlimed) the corresponding yields were high. This correlation may be connected with the trailing habit of

Lathyrus, which allows grasses and weeds to grow through it instead of crowding them out. It is also possible that the considerable extra supply of available nitrogen produced by the heavy leguminous crop encourages the growth of the other groups, thus pushing up the total yield. Apart from this, variations in yield and botanical composition seem to be entirely independent, at least as far as the three main groups are concerned.

(2) *Seasonal variation in the proportion of individual species.*

The influence of season on the relative proportion of the species in the mixed herbage of grassland presents a most complicated problem on account of the number of factors involved. Rainfall, temperature, sunshine, manuring, soil reaction and plant competition are probably but a few of the factors whose interplay determines the annual variation in the botanical composition of the herbage. In the space here available it would be an impossible task to disentangle the reasons for the seasonal changes in any detail, but an attempt will be made to correlate the major variations with the conditions in particular years.

On grassland carrying a number of diverse species growing in association, an increase in the proportion of any one species is necessarily at the expense of a decrease in one or more others. If species were all affected equally by season their relative proportion in the constitution of the herbage would remain constant. As, however, conditions that favour one plant discourage another the percentage composition of the herbage is in a continuous state of flux. Seasonal changes, therefore, resolve themselves into variations in the balance of competition from year to year. The competition between the species constituting grassland herbage is acute, but the fact that any one species is predominant under a particular set of conditions does not necessarily mean that it is in itself of necessity specially favoured by these conditions. For instance, if two species *A* and *B*, or *B* and *C* are in competition,

(a) *A* may be specially favoured by the particular conditions obtaining, while *B* is indifferent, in which case *A* will tend to predominate while *B* is reduced in quantity;

(b) *B* may be indifferent to the conditions, whereas *C* is adversely affected, in which case *B* will increase and *C* will tend to be crowded out.

This affords an explanation of the variation in the relative prevalence of certain species under different conditions of competition. *Rumex acetosa*, for instance, when grown alone gave the largest crops in soil well supplied with lime, whereas in the same experiment under conditions of

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competition it was crowded out on well-limed soils, but held its own on acid soil which was less favourable to the growth of the other competing species. In any season climatic conditions are uniform over all the plots while manurial treatments and soil reaction vary considerably, whereas from year to year the meteorological conditions fluctuate widely while the soil conditions and treatment of individual plots remain practically constant. It would be surprising, therefore, if a simple response to these changes were manifested and if seasonal variations in individual species were parallel on all the plots. What actually happens is that in average seasons the proportion of any species tends to alter irregularly, rising with one treatment and falling with another. In abnormal seasons, however, the influence of treatment may be outweighed by climate, and certain species may behave more or less uniformly regardless of manuring.

Seasonal variations in the proportion of any species may either be sudden and temporary, or gradual and progressive. In the first case wide fluctuations may occur almost every year, or a species which usually shows little response to season may suddenly increase, falling back to its usual level in the succeeding year. In the second case the tendency is for slight changes to occur over a period of years to a maximum or minimum as the case may be, followed either by a reversal and a gradual change in the other direction, or by a sudden change due to an abnormal season. The latter phenomenon is then usually succeeded by a further cycle of gradual change. Both forms of response may be manifested by different species on the same plot.

On completely manured plots, where comparatively few species occur, the range of seasonal variation is large. Where sulphate of ammonia is applied (plot 9) considerable fluctuations are apt to occur even with species that are normally present in small amount, but with nitrate of soda (plot 14) these less abundant species are much more stable. This does not apply so consistently where nitrate of soda is used alone (plot 17). With no manure (plot 3) or with minerals (plot 7) the large number of species brings the relative proportions to a lower level. On the whole the major seasonal variations again occur in the more abundant species, but there are instances of relatively large fluctuations in comparatively insignificant components of the herbage.

At the risk of being categorical it is necessary to deal with each main species separately, as the responses to season and to liming are so individual that no satisfactory grouping is possible.

Agrostis vulgaris et alba (Tables II and VII). In the absence of nitrogen the seasonal variation is comparatively small and tends to run

Table II.
Percentage of grasses in Park Grass herbage.

Plot ...	3		5 ¹	5 ²	7		9	
	U.	L.	U.	U.	U.	L.	U.	L.
<i>Agrostis vulgaris et alba.</i>								
1919	8	2	4	8	5	2	12	2
1921	25	2	13	20	12	5	27	4
1922	24	4	22	15	19	5	16	4
1923	21	3	n.	n.	15	5	23	2
1924	18	3	12	16	14	1	31	3
1925	19	2	n.	n.	11	2	17	2
1926	18	2	24	21	n.	n.	25	3
<i>Alopecurus pratensis.</i>								
1919	0.3	0.6	0.7	11	2	15	0.7	26
1921	0.7	5	1	6	1	12	0.6	22
1922	3	3	5	20	1	11	14	28
1923	0.2	7	n.	n.	2	7	0.3	28
1924	4	9	0.5	6	2	10	0.5	45
1925	1	4	n.	n.	1	9	2	42
1926	2	3	0.6	13	n.	n.	0.1	24
<i>Anthoxanthum odoratum.</i>								
1919	7	3	12	5	4	0.5	5	1
1921	4	0.6	9	4	5	0.7	25	2
1922	0.9	0.2	0.5	0.4	2	0.1	8	0.4
1923	4	1	n.	n.	4	0.8	43	1
1924	4	0.3	4	0.6	1	0.2	22	0.4
1925	7	0.5	n.	n.	2	0.1	13	0.7
1926	3	0.4	12	5	n.	n.	16	1
<i>Arrhenatherum avenaceum.</i>								
1919	0.3	0.5	2	2	3	47	47	
1921	0.3	0.2	8	3	1	5	4	43
1922	0.5	0.8	2	4	1	4	11	30
1923	0.1	0.1	n.	n.	1	3	8	35
1924	—	0.1	1	3	2	4	22	32
1925	0.2	0.4	n.	n.	5	27	20	45
1926	0.1	0.4	5	8	n.	n.	6	50
<i>Avena pubescens.</i>								
1919	4	19	0.2	2	3	9	—	—
1921	3	18	0.2	2	3	12	—	—
1922	3	11	0.3	0.7	2	5	—	—
1923	4	18	n.	n.	3	6	—	—
1924	3	16	0.3	2	1	5	—	—
1925	6	32	n.	n.	1	8	—	—
1926	4	19	0.3	2	n.	n.	—	—
<i>Dactylis glomerata.</i>								
1919	8	7	9	7	22	19	3	7
1921	12	8	14	2	14	12	1	5
1922	8	8	9	7	12	10	4	8
1923	4	3	n.	n.	10	5	0.7	2
1924	4	4	6	3	12	10	0.1	2
1925	7	6	n.	n.	34	23	0.5	4
1926	5	8	8	5	n.	n.	0.4	8

U.=unlimed; L.=limed; n.=no analysis; —=missing or below 0.1 %.

No analysis of these plots was made in 1920.

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in the same direction over a period of years, even when the percentage present is high. Sulphate of ammonia (plots 9 and 18) induces considerable annual fluctuations, completely obliterating any regular progression of change. This does not apply to the limed part of plot 9 where the quantity is very small and stable throughout. The influence of sulphate of ammonia seems to be very persistent, for on the areas on which its application has been discontinued since 1897 the annual fluctuations still occur, though they are becoming less marked where minerals are now given (plot 5²) than on the unmanured section (plot 5¹).

No uniform parallel response of *Agrostis* occurred in any particular season, but there was a tendency towards large amounts in 1921 and 1924. With farmyard manure applied alone there has been a steady slow fall since 1921, but the addition of minerals has induced a slight rhythmic rise and fall. In neither case can a correlation with type of season be traced.

Alopecurus pratensis (Tables II and VII). *Alopecurus* usually shows little response to season even where it is present in abundance, except on the limed half of plot 9. The outstanding exception to this was in 1922 when a sudden increase in quantity occurred on many plots, even where the amount present was usually negligible. With ammonium salts and minerals (plot 9) the rise was from 0.6 to 13.8 per cent.; again the persistent influence of ammonium salts was manifest, as on plot 5¹ the rise was from 1.0 to 4.6 per cent. and on plot 5² from 5.6 to 20.2 per cent. With nitrogen as nitrate of soda the annual fluctuation is not proportionally very great, but again a big increase occurred in 1922. With dung, with and without minerals, a rhythmic variation occurred, with a tendency to high percentages in 1922.

The use of lime with sulphate of ammonia and minerals has induced much larger and more irregular annual variations, but the increase in 1922 did not occur with this combination of fertilisers.

The climatic conditions in 1922 were not in themselves abnormal, exhibiting a fairly high rainfall, plenty of sunshine and rather low mean temperature. In 1921, however, severe drought persisted throughout the year, affecting the aftermath so seriously that no second crop could be cut. On certain plots, notably those receiving past or present treatment with ammonium sulphate without lime, so much damage was done that the succeeding 1922 crop was abnormally low, and it was on these plots that the proportion of *Alopecurus* rose most. This affords further evidence of the comparative indifference of *Alopecurus* to diverse types of season, as the adverse conditions of drought reduced it so much less than other

species that its proportion in the herbage was spectacularly increased. On the limed part of plot 9, and on other plots where the yield was not seriously depressed, the proportion of *Alopecurus* remained unaffected.

This indifference of *Alopecurus* to season is of special interest in view of its sensitiveness in other respects, as poverty and acidity reduce it to a low ebb, whereas heavy feeding and lime encourage it greatly.

Anthoxanthum odoratum (Tables II and VII). Sufficient quantities to show significant response to season only occur in the presence of sulphate of ammonia and minerals without lime. The yearly variation was considerable between 1919 and 1924, after which the proportion became fairly stabilised till 1929. In that year extreme winter frost was followed by three months of exceptionally low rainfall from January to March, and the herbage on the unlimed sulphate of ammonia plots was practically killed. As recovery took place in succeeding years *Holcus lanatus* dominated the situation, *Anthoxanthum* being practically suppressed. The species was exceptionally abundant in 1923, rising from 8.4 to 42.6 per cent. and from 2.6 to 19.7 per cent. on plots 9 and 18 respectively, falling again heavily in 1924. The most characteristic feature of 1923 was its scarcity of bright sunshine associated with a low mean temperature during the later half of the growing season. The total bright sunshine from January to June was only 587 hours, compared with an average of 784 hours during the ten years 1918–28.

Arrhenatherum avenaceum (Tables II and VII). Good manuring, particularly with lime, is essential to the well-being of this species. With nitrogenous and mineral manures (plots 9 and 14) the annual fluctuations in percentage are often relatively large, but it is difficult to link up the variations with special types of season. Without lime the proportion with ammonium sulphate is usually low, but in 1919 it rose to the high figure of 47 per cent., in a year characterised during the first three months by abnormally high rainfall, low sunshine and low mean temperature. With lime, and with nitrate of soda, this increase did not occur, indicating some correlation between the influence of soil acidity and the particular seasonal conditions. With minerals, season has little effect and the increase to 28 per cent. in 1925 is noteworthy in that it only occurred in the presence of lime, whereas without lime *Arrhenatherum* was unable to take advantage of the opportunity for increase offered by the sudden drop in the proportion of *Lathyrus*.

With dung the fluctuations show no definite correlation with season, but with minerals also (plot 20) rhythmic changes occur comparable with those of the grasses as a whole.

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Avena pubescens (Tables II and VIII). Although rarely present in any great quantity, *Avena* is outstanding in its persistent regularity in proportion from year to year, particularly where no lime has been applied. With lime slightly more variation occurs, but there is a definite tendency towards stabilisation of proportion.

Bromus mollis has always been recognised as being most variable with season. Frequently it is quite insignificant on all the plots on which it occurs, while in other years it may suddenly increase, far more on certain plots than on others. In 1903 *Bromus* constituted 23 per cent. of the herbage with heavy nitrate and minerals, whereas in 1919 it was only 0.5 per cent. During the period of annual analyses *Bromus* at first was very insignificant, being less than 1 per cent. on any plot, but the years 1921, 1922 and 1923 were favourable to its growth on plots 7, 14 and 20 (Table III).

Table III.

Percentages of *Bromus mollis* in Park Grass herbage.

	Plot 7		Plot 14		Plot 20		
	U.	L.	U.	L.	U.	L.L.	H.L.
1920	No analysis		0.5	0.2	0.3	0.1	0.1
1921	0.1	2.6	1.0	1.5	0.5	1.2	0.7
1922	0.2	5.4	4.6	6.5	1.7	2.2	2.8
1923	0.1	7.9	3.7	15.2	2.4	2.4	2.9
1924	—	—	—	—	—	0.03	—

U. = unlimed; L. = limed; L.L. = light limed; H.L. = heavy limed.

With minerals (plot 7) there was no increase without lime, but a progressive rise with lime, whereas with minerals together with nitrate of soda or dung the improvement was manifest also in the absence of lime. After 1923 the species reverted to insignificance and no marked increase has since been recorded.

Dactylis glomerata (Tables II and VIII). Seasonal variations are usually slight, and if for any reason a big change does occur the tendency is for the proportion to remain at the new level in future years. In 1925 with minerals (plot 7) and with nitrate (plot 17) *Dactylis* responded favourably to the conditions which reduced *Lathyrus*, and (on plot 7) showed only a gradual decrease over the next eight years, with no seasonal drop even with the unfavourable conditions of frost and drought in 1929. The familiar rhythmic cycle was shown on both dunged plots, the highest maxima being reached in 1919 and 1927.

*Festuca ovina*¹ (Tables IV and VIII). The behaviour of *Festuca* was

¹ Throughout this paper *Festuca ovina* is used in a group sense, including also *F. rubra* and *F. duriuscula*, as in Bentham and Hooker's *Handbook of the British Flora*.

comparatively uniform over a wide range of manuring. In nearly all cases a steady increase in percentage occurred from 1919 to 1923, followed by a sudden drop in the next year, when many other results were erratic. The only exception was on the plots on which ammonium salts had been discontinued, where *Festuca* remained at a high level, notably on the area now left unmanured. Apparently *Festuca* is usually less affected by season than by other environmental factors, though occasionally a particular combination of meteorological conditions may induce a direct response which, however, is still closely influenced by manuring and soil conditions.

Table IV.

Percentage of grasses in Park Grass herbage.

Plot ...	3		5 ¹	5 ²	7		9	
	U.	L.	U.	U.	U.	L.	U.	L.
<i>Festuca ovina.</i>								
1919	7	5	46	20	7	5	4	6
1921	13	13	40	28	18	10	10	8
1922	13	10	15	17	24	13	11	5
1923	20	18	n.	n.	28	10	12	13
1924	10	8	38	20	11	2	8	3
1925	11	8	n.	n.	9	3	4	2
1926	8	6	28	14	n.	n.	2	3
<i>Holcus lanatus.</i>								
1919	9	8	0.6	1	4	2	12	0.8
1921	11	9	0.3	2	12	2	30	2
1922	3	2	0.3	1	4	2	32	1
1923	4	3	n.	n.	2	1	12	0.2
1924	2	2	0.2	0.1	1	0.5	14	0.2
1925	8	5	n.	n.	6	0.7	40	0.3
1926	7	6	1	4	n.	n.	51	3

U. = unlimed; L. = limed; n. = no analysis.

Holcus lanatus (Tables IV and VIII). *Holcus* is probably more directly responsive to season than any other species present. This may be partly due to its habit of starting into growth early in the year, when plots containing much *Holcus* are green while all others are still brown and lifeless. With moderate manuring the quantity is usually small, marked increases occurring in 1921, 1925 and 1927, when the seasons were relatively warm. On the acid soil produced by sulphate of ammonia and minerals (plot 9) *Holcus* has of recent years come to dominate the situation. In 1919, 1923 and 1924 it was depressed to below 15 per cent., the common characteristic of these years being low temperatures in either the early or later part of the growing season. After this, the proportion having increased to 75 per cent. during a series of favourable years, the whole herbage was wiped out by the frost and drought of 1929.

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The earliness of *Holcus* then gave it an advantage and it returned 100 per cent. strong to the exclusion of all else. In 1931, again a year of low temperatures and rainfall during the first quarter, a certain amount of *Festuca* and *Agrostis* reasserted themselves and *Holcus* was reduced to 76 per cent., but this did not last and since then the entire herbage has consisted of *Holcus* for three successive years. This increasing dominance of *Holcus* cannot be associated with increasing acidity, as the soil reaction has remained unchanged at 4.0 since 1903. It is rather a question of increasing competition, as *Holcus* does not mind acidity while the other species tend to be adversely affected. If *Holcus* is benefited by a favourable season it is able to maintain its improved position because

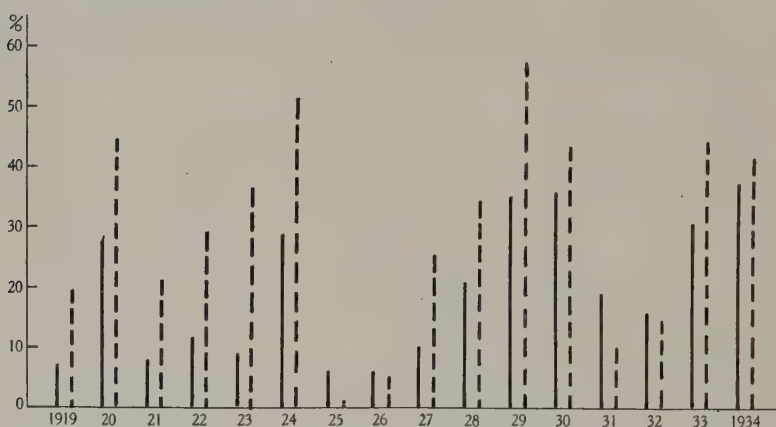


Fig. 4. Percentage of *Lathyrus pratensis* on plot 7 (minerals), 1919-34. — Unlimed. - - - - Limed. (For 1920, and 1926-34 the figures are those for total Leguminosae, of which nearly all was *Lathyrus*.)

the other species are less able to compete effectively and regain their lost ground. It remains to be seen whether further reduction of *Holcus* in unfavourable seasons will ultimately result in a more balanced herbage on the acid plots.

Lathyrus pratensis (Tables V and IX). With continuous mineral manuring (plot 7) *Lathyrus* exhibits more spectacular fluctuations than any other species on the plot. It forms by far the largest proportion of the leguminous plants, especially on the unlimed area, and although complete analyses were only made till 1925 the Leguminosae from the later partial analyses give a true picture of the behaviour of *Lathyrus*. Prior to 1925 the proportion had steadily risen, rather irregularly to 28 per cent. without lime, and fairly steadily to 51 per cent. with lime,

but in this year the figures dropped to 6 and 1 per cent. respectively (Fig. 4). It is difficult to associate this sudden reduction with any particular seasonal peculiarity, as all the months of the growing season were more or less of an average nature. A second year's depression was followed by a rhythmical rise and fall with maxima in 1929 and 1933, the rate of change being greater in the presence of lime. This response to season was paralleled on the plots receiving dung, where the effect was again most marked with lime.

Centaurea nigra (Table V). On most plots this is not plentiful, but where it occurs in any quantity it shows considerable response in certain seasons, the years 1922 and 1924 being very favourable. In 1922 after a preceding year of drought the increase of *Centaurea* was noticeable wherever it occurred, except with dung, but in 1924 the response was less widespread, being confined to plots without manure or with minerals after complete fertilisers (plots 3, 5²).

Plantago lanceolata (Tables V and IX). From the data available it does not appear that *Plantago* shows any striking variation with season, though the fluctuations are greater on the mineral plots (7 and 15) than on those receiving no manure (plot 3) or nitrate of soda (plot 17). Unfortunately plots 15 and 17, on which the species is most abundant, have only been analysed every other year, and it is therefore impossible to obtain an accurate estimate of response in these cases. The periodic variation in proportion is usually greater on the unlimed areas of the plots.

Table V.

*Percentages of leguminous and miscellaneous plants
in Park Grass herbage.*

Plot	...	<i>Lathyrus pratensis</i>			<i>Centaurea nigra</i>					<i>Plantago lanceolata</i>	
		5 ²		7	3		5 ¹	5 ²	7	3	
		U.	U.	L.	U.	L.	U.	U.	U.	U.	L.
1919		1	7	20	6	6	4	3	3	19	12
1921		6	8	21	4	5	7	3	3	8	9
1922		2	12	29	7	21	19	7	6	11	8
1923		n.	9	36	2	4	n.	n.	1	11	7
1924		11	28	51	9	9	14	6	0.5	10	5
1925		n.	6	1	3	3	n.	n.	2	15	7
1926		7	n.	n.	3	4	6	3	n.	17	9

U. = unlimed; L. = limed; n. = no analysis.

Rumex acetosa (Table IX). The quantity is usually small and relatively stable except in a few cases where manurial treatment has changed, when an occasional year of abundant *Rumex* has occurred. With complete

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fertilisers without phosphate (plot 18) the years 1919 and 1926 were favourable, otherwise response to season is not very marked in most years.

B. RATE OF CHANGE DUE TO LIMING.

The system of liming instituted in 1903 has demonstrated fully the radical change in the constitution of grassland herbage brought about by the addition of lime to other fertilisers. As, however, complete botanical analyses were not made till eleven years afterwards, no quantitative information was gained as to the rate of response of different species. A few plots which had previously been omitted from the scheme have since been dressed with lime from 1920 onwards, and the changes in yield and botanical composition investigated. The effect on yield has been discussed previously (3, 4), and the analyses now under consideration indicate the response of the component species to the change in conditions.

(1) *Response of main groups of grasses, leguminous and miscellaneous plants to liming.*

With complete manures, whether supplied as dung or artificial fertilisers, the balance of the three main groups was not affected by liming, the variations from year to year being quite irregular, but with one-sided manures a definite bias in one direction appeared sooner or later. With minerals after nitrate of soda (plot 15) liming caused decrease in the proportion of miscellaneous plants, which set in immediately, and an increase in the leguminous plants after a single year of depression, the differences due to liming often being very considerable. This is parallel to the effect of lime on plot 7 which has always received minerals. The behaviour of the grasses for several years was more erratic, but since 1927 the proportion on the limed area has definitely been the lower. It is possible that the grasses are less influenced than the other two groups, and that the balance between the latter determines the relative proportion of grasses on the limed and unlimed areas.

With nitrate of soda alone (plot 17) the interplay was almost entirely between the grasses and miscellaneous plants, as the Leguminosae were merely raised by liming from a negligible amount to a maximum of 2 per cent. Here the issue was clear-cut from the first, grasses being improved and the miscellaneous species discouraged consistently every year.

It appears, therefore, that alterations caused by liming in the proportions of the main groups usually manifest themselves at once, though the effect may become accentuated with time. It is also possible for one

or more groups to be consistently affected, and for either or both the others to show no direct response for several years, if at all.

(2) *Response of individual species to liming.*

Although liming may not have any definite influence on the proportion of the three main groups with certain combinations of fertilisers, the relative percentage of the individual species within those groups may be considerably affected.

The analyses show that while some species respond to liming as soon as the first application is made, others may remain comparatively unaffected for several years or even till the second dressing is given. Very occasionally the primary direction of response may be reversed after the later applications. For purposes of reference in considering these changes the available pH value of the soils may be of interest (5).

Table VI.

pH value of Park Grass soils. Lime applied 1920, 1924, 1928, 1932.

	Plot	U.	L.		Plot	U.	L.L.	H.L.
1923	14	6.38	6.74	1923	18	4.46	4.69	5.22
	15	5.54	6.13		19	5.75	5.71	6.22
	17	6.31	6.81		20	6.00	6.15	6.58
1931	17	5.9	6.5					
1933	17	6.0	7.2					

U. = unlimed; L. = limed; L.L. = light limed; H.L. = heavy limed.

Agrostis vulgaris (Table VII). This was consistently reduced by adequate liming, though in the first years the reduction was often small, sometimes increasing after later applications (plots 15 and 18). With dung (plot 19) the lighter dressing of lime was insufficient to cause any reduction even after the third application, but where artificials were also present decrease occurred after the second dressing.

Alopecurus pratensis (Table VII). The response to liming was closely associated with the manuring, showing increase with sulphate of ammonia and minerals and with minerals alone, decrease with nitrate of soda and minerals, while no consistent behaviour was evidenced in the presence of dung. With nitrate of soda alone no effect was shown for at least eight years, when a possible slight depression set in. The general tendency was for the response in either direction not to be evident for at least two years after the first application of lime, except with minerals alone when no delay occurred. In several seasons the lighter dressings of lime induced the largest proportions of *Alopecurus* in the presence of sulphate of ammonia and minerals.

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Table VII.

Percentage of grasses in Park Grass herbage.

Plot...	14		15		17		18			19			20		
	U.	L.	U.	L.	U.	L.	U.	L.L.	H.L.	U.	L.L.	H.L.	U.	L.L.	H.L.
<i>Agrostis vulgaris et alba.</i>															
1919	0.5	n.	11	n.	6	n.	18	n.	n.	7	n.	n.	7	n.	n.
1920	—	0.2	n.	n.	n.	n.	43	45	35	18	15	15	11	15	6
1921	—	1	15	9	6	5	51	41	42	22	18	14	13	10	5
1922	0.2	0.8	n.	n.	n.	n.	44	36	31	13	16	10	10	11	5
1923	—	0.6	13	14	9	4	48	28	27	14	15	10	10	13	5
1924	0.3	0.3	n.	n.	n.	n.	72	37	26	13	18	8	15	9	4
1925	0.3	—	17	8	3	2	63	37	14	11	10	3	14	4	3
1926	n.	n.	n.	n.	n.	n.	47	18	12	9	9	3	10	5	4
1927	n.	n.	12	6	6	4	75	22	10	5	5	2	4	3	2
1928	n.	n.	n.	n.	n.	n.	59	18	5	6	5	2	4	4	1
<i>Alopecurus pratensis.</i>															
1919	54	n.	30	n.	13	n.	5	n.	n.	22	n.	n.	30	n.	n.
1920	48	53	n.	n.	n.	n.	6	4	6	16	15	22	27	22	30
1921	34	33	8	20	12	10	3	5	5	13	20	16	19	22	31
1922	58	42	n.	n.	n.	n.	10	25	17	22	20	19	23	24	33
1923	42	24	8	15	13	10	5	10	8	16	16	15	29	17	25
1924	44	29	n.	n.	n.	n.	5	23	22	17	16	17	16	27	21
1925	36	19	10	28	14	13	4	14	14	16	22	24	17	15	19
1926	n.	n.	n.	n.	n.	n.	6	25	25	29	30	27	27	22	26
1927	n.	n.	9	14	14	14	4	22	19	26	28	17	30	19	23
1928	n.	n.	n.	n.	n.	n.	5	46	23	33	43	21	46	31	24
<i>Anthoxanthum odoratum.</i>															
1919	0.4	2	3	n.	7	n.	3	n.	n.	4	n.	n.	1	n.	n.
1920	—	—	n.	n.	n.	n.	6	7	5	5	4	2	0.8	2	0.7
1921	—	0.6	5	2	9	3	8	4	4	10	8	3	1	6	2
1922	—	—	n.	n.	n.	n.	3	0.7	1	4	2	0.6	0.4	1	0.3
1923	—	0.1	4	2	7	3	20	2	3	12	9	1	0.3	6	1
1924	—	—	n.	n.	n.	n.	4	0.1	—	4	7	0.6	1	1	0.2
1925	—	—	3	1	7	0.9	3	0.1	—	10	5	0.8	1	2	0.4
1926	n.	n.	n.	n.	n.	n.	4	0.1	0.1	8	8	0.7	1	4	1
1927	n.	n.	4	0.9	11	3	3	0.1	—	9	9	0.5	0.6	3	0.8
1928	n.	n.	n.	n.	n.	n.	7	0.5	—	12	8	0.7	2	6	0.7
<i>Arrhenatherum avenaceum.</i>															
1919	23	n.	1	n.	0.3	n.	2	n.	n.	8	n.	n.	5	n.	n.
1920	37	30	n.	n.	n.	n.	0.5	0.8	0.5	1	4	5	6	2	2
1921	48	41	3	4	—	—	0.7	2	2	8	0.3	14	10	10	4
1922	25	25	n.	n.	n.	n.	1	2	3	11	2	5	7	6	3
1923	33	35	1	3	—	0.4	0.1	2	3	9	2	7	6	12	2
1924	39	40	n.	n.	n.	n.	0.3	2	10	11	1	8	6	10	0.4
1925	40	54	1	1	0.5	—	1	7	1	19	3	15	9	33	4
1926	n.	—	n.	n.	n.	n.	2	9	11	12	6	22	18	24	8
1927	—	—	9	5	1	2	2	10	19	18	6	22	25	31	7
1928	n.	n.	n.	n.	n.	n.	0.4	3	18	8	3	16	11	9	4

U.=unlimed; L.=limed; L.L.=light limed; H.L.=heavy limed;
n.=no analysis; —=missing or below 0.1 %.

The behaviour of *Alopecurus* is closely associated with soil reaction. With low pH, as on plot 18, pH 4.6, and plot 15, pH 5.54, liming is beneficial to the proportion of the species. As neutrality is approached the benefit disappears (plot 17, pH 6.31; plot 19, pH 5.75; plot 20, pH 6.00), or an adverse response occurs (plot 14, pH 6.38) giving results which exactly parallel those obtained by earlier treatment of the other plots on the area.

Anthoxanthum odoratum (Table VII). This is very impatient of adequate dressings of lime and in all cases showed an immediate reduction, rapidly being decreased to an almost negligible quantity. Lighter dressings were somewhat slower in action with sulphate of ammonia and minerals, though they eventually had the same effect, but on the dunged plots the smaller quantity of lime was insufficient to bring about any reduction.

Arrhenatherum avenaceum (Table VII). This is so remarkably inconsistent in its behaviour that it is probable that the action of lime in any season is often profoundly influenced by other factors. With nitrate of soda and minerals (plot 14) a depression at the beginning due to liming was gradually converted into a beneficial effect in later years, but as no analyses have been made since 1925 it is unknown whether this has persisted. At the present time, however, *Arrhenatherum* forms the major part of the herbage on both the limed and unlimed areas of this plot, so that it is unlikely that any very striking effect of liming is manifest with this species.

With sulphate of ammonia and minerals the quantity was originally very small, but a gradual increase was induced by liming, until after the second application the proportion rose to 5 per cent. with the light dressing and to 19 per cent. with the heavy dressing. With dung the result is almost inexplicable. With heavy dressings (on plot 19) the response was irregular, either increase or decrease occurring with season, but from the beginning the lighter dressings have been adverse, reducing the proportion of *Arrhenatherum* much below that on the unlimed or heavy-limed sections. With the addition of artificials to dung (plot 20) the heavier lime dressings have been consistently adverse, whereas the lighter dressings have frequently given the best result of all. This was specially so in 1925, when the percentage rose to 33 with light lime compared with 9 and 4 per cent. on the other sections.

Avena pubescens (Table VIII). Liming proved very beneficial as a general rule, the increase usually beginning at once, and becoming more marked after a second application. With nitrate of soda and minerals lime had no effect, and on the dunged plots, also, the lighter dressings were inadequate and induced no response.

Dactylis glomerata (Table VIII). With nitrate of soda lime definitely prejudices the growth of *Dactylis*, the greatest reduction being with nitrate alone. With minerals and ammonium salts, on the contrary, it is much encouraged by lime, though the benefit was not manifested until after the second application (Fig. 5). With dung no response has occurred,

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except for an occasional tendency to an increased proportion with the lighter dressing.

Festuca ovina (Table VIII). Liming had no consistent effect on *Festuca* except with nitrate of soda, when it caused an immediate and marked increase. A similar result had previously been obtained with the same amount of nitrate together with minerals. With the double dressing of nitrate and minerals (plot 14) the negligible amount of *Festuca* remained uninfluenced, possibly because of undue competition from the larger species of grass encouraged by this manuring.

Holcus lanatus (Table VIII). Liming caused a general tendency to reduction throughout, except on plot 20 when it was quite ineffective. On plot 18 (ammonium salts and minerals) the effect was not a true

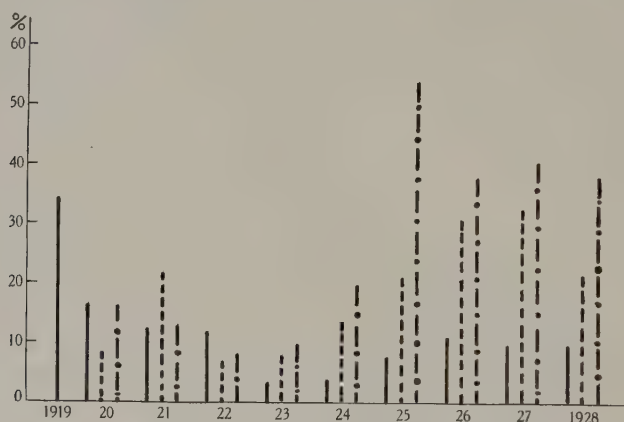


Fig. 5. Percentage of *Dactylis glomerata* on plot 18 (ammonium salts and minerals without phosphate), 1919-28. — Unlimed. - - - - - Light limed. - · - · - Heavy limed.

reduction but was due to the failure of the species, in the presence of lime, to respond to favourable environmental conditions after the abnormal season of 1924, whereas on the unlimed area the proportion increased considerably.

Poa pratensis. This was primarily insignificant or absent on all plots, but with ammonium salts and minerals (plot 18) it showed an immediate and progressive increase with lime, responding equally to both light and heavy dressings, reaching a maximum of 7 per cent. in some years.

Lathyrus pratensis (Table IX). No consistent response to liming was shown except with mineral manures where it was benefited immediately. The difference in the proportion of *Lathyrus* with and without lime fluctuates widely from year to year, as in 1927 the variation was from 5 per cent. unlimed to 26 per cent. limed, whereas in 1923 there was a

Table VIII.
Percentages of grasses in Park Grass herbage.

Plot ...	14		15		17		18			19			20		
	U.	L.	U.	L.	U.	L.	U.	L.L.	H.L.	U.	L.L.	H.L.	U.	L.L.	H.L.
<i>Avena pubescens.</i>															
1919	4	n.	2	n.	5	n.	0.1	n.	n.	3	n.	n.	10	n.	n.
1920	0.2	1	n.	n.	n.	n.	—	—	—	3	4	5	11	10	14
1921	2	4	3	3	4	7	—	—	—	4	2	5	12	8	19
1922	0.7	2	n.	n.	n.	n.	0.3	0.4	1	2	1	3	8	5	7
1923	0.8	5	2	3	3	10	—	0.1	—	2	3	4	8	5	12
1924	0.4	3	n.	n.	n.	n.	—	—	—	1	2	6	5	5	10
1925	0.3	0.1	2	6	2	16	—	—	—	2	3	7	6	3	16
1926	n.	n.	n.	n.	n.	n.	—	0.3	1	2	3	7	6	7	16
1927	n.	n.	3	11	3	17	—	0.1	0.2	3	4	11	5	8	24
1928	n.	n.	n.	n.	n.	n.	—	—	—	3	3	12	7	9	29
<i>Dactylis glomerata.</i>															
1919	3	n.	5	n.	8	n.	34	n.	n.	16	n.	n.	12	n.	n.
1920	5	3	n.	n.	n.	n.	17	8	16	11	9	9	10	8	9
1921	5	3	5	2	5	11	12	22	13	6	16	9	6	9	9
1922	3	2	n.	n.	n.	n.	12	7	8	5	15	12	9	11	8
1923	5	0.6	3	2	7	4	3	8	10	5	6	4	6	4	3
1924	3	1	n.	n.	n.	n.	4	13	19	7	4	5	6	5	5
1925	7	3	10	6	28	15	8	21	53	9	21	18	10	13	9
1926	n.	n.	n.	n.	n.	n.	11	31	37	14	15	16	11	14	13
1927	n.	n.	16	5	24	7	10	32	40	17	26	17	15	18	14
1928	n.	n.	n.	n.	n.	n.	9	21	38	10	11	9	7	7	6
<i>Festuca ovina.</i>															
1919	5	n.	7	n.	4	n.	4	n.	n.	6	n.	n.	4	n.	n.
1920	—	2	n.	n.	n.	n.	14	5	12	12	12	15	10	10	10
1921	0.1	5	22	20	12	21	12	6	7	12	10	15	9	9	7
1922	0.2	5	n.	n.	n.	n.	9	10	12	18	15	15	16	14	13
1923	0.2	9	21	23	17	35	18	26	31	13	15	17	14	15	21
1924	0.1	7	n.	n.	n.	n.	5	6	4	8	10	13	8	6	10
1925	—	0.1	9	10	6	22	6	5	5	8	5	7	4	1	4
1926	n.	n.	n.	n.	n.	n.	3	2	2	4	3	4	3	3	5
1927	n.	n.	8	8	5	21	3	3	3	6	3	11	3	3	5
1928	n.	n.	n.	n.	n.	n.	6	3	3	8	4	14	5	6	9
<i>Holcus lanatus.</i>															
1919	—	n.	6	n.	11	n.	2	n.	n.	2	n.	n.	7	n.	n.
1920	—	—	n.	n.	n.	n.	1	8	2	2	2	2	3	5	7
1921	—	—	11	5	16	12	4	3	5	5	7	2	10	10	8
1922	—	—	n.	n.	n.	n.	0.9	2	1	1	2	0.5	3	4	3
1923	—	—	2	2	3	3	0.2	1	2	3	2	0.2	2	2	2
1924	—	—	n.	n.	n.	n.	0.2	0.2	—	1	2	0.8	2	2	1
1925	—	—	10	4	10	6	0.8	0.4	0.3	6	2	2	4	4	5
1926	n.	n.	n.	n.	n.	n.	3	1	2	4	2	1	7	7	8
1927	n.	n.	12	3	12	10	2	3	2	3	2	1	7	7	7
1928	n.	n.	n.	n.	n.	n.	8	0.8	2	5	3	0.9	4	8	5

U. = unlimed; L. = limed; L.L. = light limed; H.L. = heavy limed;
n. = no analysis; — = missing or below 0.1 %.

slight but transitory depression on the limed areas. With dung and artificials the lighter dressings encouraged *Lathyrus* more than the other two treatments, but this was merely temporary and disappeared as soon as the second dressing of lime was applied.

Trifolium repens. This is usually present only in small amount in the first cut of hay, as its tendency is to develop rather late. In 1923, 1929 and 1933 unusual quantities occurred on the limed part of plot 15, with

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minerals, reaching the high figures of 19 per cent. in 1929, a year of abnormal seasonal conditions. This year, also, large amounts were recorded from the dunged plots with lime, though this increase did not appear on any other occasion. Owing to the late habit of the species it is difficult to ascertain the true response of *Trifolium repens* to lime from the data available.

Achillea millefolium (Table IX). The amount was only significant with minerals, the effect of lime being to keep the proportion constant, preventing it from responding to any seasonal conditions that were advantageous to the species, as occurred in 1929.

Table IX.

*Percentages of leguminous and miscellaneous species
in Park Grass herbage.*

	<i>Lathyrus pratensis</i>									<i>Rumex acetosa</i>		
Plot...	15		19			20			18			
	U.	L.	U.	L.L.	H.L.	U.	L.L.	H.L.	U.	L.L.	H.L.	
1919	5	n.	6	n.	n.	5	n.	n.	24	n.	n.	
1920	n.	n.	12	18	8	4	15	5	9	15	18	
1921	8	18	5	2	3	3	5	4	6	11	14	
1922	n.	n.	7	6	7	1	8	4	4	6	8	
1923	15	11	7	9	15	2	10	5	2	13	12	
1924	n.	n.	19	18	21	22	17	30	2	10	10	
1925	4	14	4	3	1	10	2	4	10	11	5	
1926	n.	n.	2	1	0.9	4	1	3	21	9	5	
1927	5	26	1	1	1	2	0.5	3	1	1	0.8	
1928	n.	n.	2	1	1	3	2	5	3	2	1	
1929	16	25	—	—	—	—	—	—	—	—	—	
1931	5	—	—	—	—	—	—	—	—	—	—	
1933	8	14	—	—	—	—	—	—	—	—	—	

	<i>Achillea millefolium</i>		<i>Plantago lanceolata</i>			
Plot ...	15		15		17	
	U.	L.	U.	L.	U.	L.
1919	5	n.	4	n.	24	n.
1921	3	2	7	4	29	18
1923	2	1	13	5	27	16
1925	7	2	15	8	17	11
1927	6	3	6	5	16	8
1929	16	4	6	4	23	15
1931	8	—	2	—	—	—
1933	1	0.9	4	10	16	17

U. = unlimed; L. = limed; L.L. = light limed; H.L. = heavy limed;
n. = no analysis; — = missing or below 0.1 %.

Plantago lanceolata (Table IX). With minerals and with nitrate of soda liming caused a marked decrease in the proportion of *Plantago*, but where the amount was originally insignificant the species remained unaffected and was not completely eradicated by lime.

Rumex acetosa (Table IX). Relatively small quantities were present on most plots and showed a general tendency to reduction with lime except with ammonium sulphate and minerals without phosphate (plot 18). In the latter case lime apparently induced a marked increase in *Rumex* for the first five years, after which a reaction set in and the effect of liming became variable with season, tending to decrease the species. Care is necessary in interpreting these results owing to the large percentage of *Rumex* that was present in 1919. In that year the sample was drawn from the whole plot, and in 1920 from the three sections as divided for liming. It is quite possible that the *Rumex* was originally distributed unevenly over the plot, being more prevalent on the areas which later received lime, and that the figures in the first few years are more or less a reflection of the original proportion of *Rumex* of the plot. The change towards a decrease with lime which set in after the second application may therefore present the real state of affairs, *i.e.* that with minerals and ammonium salts lime tends to decrease the proportion of *Rumex*. If this be true, then the reaction corresponds with the earlier results on the other plots with similar manuring.

Shade is another factor which influences the balance of species in herbage, as is shown by a section of the limed area of plot 14 (nitrate and minerals) which is in the shadow of a large oak tree during the earlier hours of the day. The proportion of the main groups is not usually much affected, as it is determined in this case by the dominating influence of the manurial treatment, though a certain increase in Leguminosae is sometimes registered. The individual species, on the contrary, show striking variations (Table X). *Festuca ovina* and *Avena pubescens* are increased by shading from a negligible quantity to about 15 per cent. in a typical year, while *Arrhenatherum* shows a correspondingly heavy drop, *Poa trivialis* also being reduced. *Alopecurus* and *Dactylis*, although present in quantity, seem to be indifferent to the effect of shading.

Table X.

Effect of shade on the botanical composition of herbage, 1925.

(Plot 14, nitrate of soda and minerals, with lime.)

	Unshaded %	Shaded %
Increased: <i>Avena pubescens</i>	0.1	16.0
<i>Festuca ovina</i>	0.1	16.9
<i>Lathyrus pratensis</i>	Trace	5.7
Indifferent: <i>Alopecurus pratensis</i>	18.9	22.0
<i>Dactylis glomerata</i>	3.0	2.7
Decreased: <i>Arrhenatherum avenaceum</i>	54.0	29.6
<i>Poa trivialis</i>	6.6	Trace

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The occasional increase in leguminous plants, such as occurred in 1920 and 1925, is entirely due to *Lathyrus pratensis*, but the proportion has never exceeded 7 per cent.

The available numerical data only refer to the one plot, but observations in the field corroborate the fact that shading exercises a marked influence on the botanical composition of herbage, though the direction of response of any individual species may vary according to soil conditions and manurial treatment.

SUMMARY.

The botanical composition of the herbage of grassland under constant manurial treatment varies considerably from year to year.

With complete fertilisers including nitrogen and minerals the relative proportions of the three main groups of species, *i.e.* grasses, leguminous and miscellaneous plants, are not usually much affected by season, though the individual species do vary, but with one-sided fertilisers and on unmanured areas wide fluctuations occur in the percentage of these groups. No correlation can be traced between the annual variations in the yield and the botanical composition of the herbage, except for some suggestion of association between high yield and high percentage of leguminous plants with long-continued mineral manuring.

The variations of individual species occur on all plots. They may be caused by direct or indirect response to season and are much influenced by the type of manuring. It is often difficult to determine whether a marked increase or decrease of a species in any year is due to climatic conditions being beneficial or detrimental to that particular species. It may be that the real effect is on other constituents of the herbage which change so much that the proportion of the species under consideration is radically affected (*cf.* *Alopecurus* in 1922). In some cases, especially with organic fertilisers, the main groups and also certain species (as *Alopecurus*, *Arrhenatherum*, *Dactylis*) show a tendency to rhythmic changes with season, rising and falling over a period of years. In other cases the fluctuations are more abrupt and irregular, sometimes being exaggerated in the presence of lime.

The application of lime to plots with long-established manurial treatment does not affect the balance of the three main groups with complete fertilisers, but with one-sided manures a definite bias in one direction appears sooner or later. Individual species usually respond to lime at once, showing a change of proportion at the first succeeding cut, but under certain soil conditions a delay may occur until a second dressing

has been given. It would appear that the maximum effect of liming is reached within a few years from the first application, after which fluctuations with season may again become more obvious.

Shade is also a factor which influences the balance of species in herbage. The available data is limited to a single plot, but indicates that certain species may be greatly increased or decreased as a result of shading, whereas the proportion of other species may not be affected.

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OBSERVATIONS ON YIELD TRIALS AND VARIETIES OF SPRING SOWN OATS IN RELATION TO DIFFERENT LEVELS OF PRODUCTIVITY IN WALES

BY E. T. JONES.

(Welsh Plant Breeding Station.)

(With 1 Chart.)

THE diversity of conditions, such as of altitude, aspect, soil fertility, rainfall and temperature, under which oats are grown in Wales presents interesting breeding and agronomic problems and at the same time confronts the investigator concerned with the breeding and testing of new varieties with many difficulties; what, for example, is the best means of arranging for an adequate and satisfactory system of yield testing, and what other criteria should be employed in order to evaluate the varieties, having regard to the diverse conditions involved?

When breeding investigations were begun at Aberystwyth in 1919 steps were taken to determine the relative merits of indigenous and other varieties of oats. A commencement was made by taking a number of convenient centres representing a range of environmental conditions, and at these a number of contrasting kinds of varieties were grown. Out of these early studies one feature of much importance emerged, namely, that from centre to centre according to the level of productivity marked changes occurred in the ranking or relative yields of the varieties. These investigations conducted chiefly by Mr Martin G. Jones¹ showed a pronounced superiority of the high tillering indigenous strains over the heavy-grained low tillering newer varieties at the centres of low productivity—as judged by the absolute yield of a standard or control variety, in most cases Record—whereas at the centres of high productivity the newer heavy-grained varieties excelled.

INDICATION OF THE DIFFERENTIAL RESPONSE OF OAT VARIETIES.

These early investigations were taken a step farther when the same investigator designed trials in which were included single selected representatives of early, medium and late ripening, low and high tillering

¹ Now a member of the Staff of Messrs Imperial Chemical Industries, Ltd., at Jealott's Hill.

varieties respectively, so constituting a trial composed of representative types of oats, in short a "Type Trial". The Type Trials were conducted at centres representing different environmental conditions. An example of the kind of result obtained in this way is shown in Chart 1 which has been taken from the *Station Bulletin*, Series C, No. 3 (2).

In this chart, it should be explained, the yield of Record, taken as 100 at each centre, is shown by the horizontal line and the proportionate grain yields of the other varieties by their positions on the ordinates as they fall either above or below the yield of Record at the

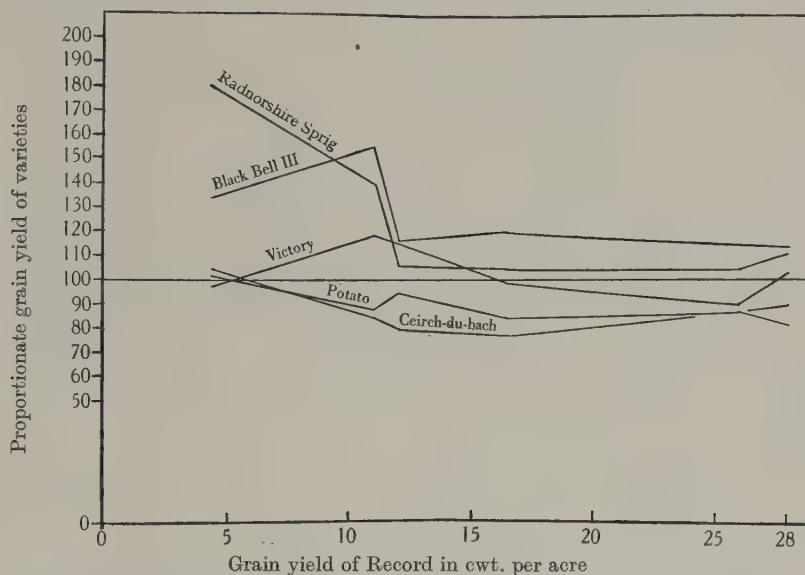


Chart 1. To contrast the grain yield of five varieties with that of Record (the standard) at six centres.

respective six centres. At these centres the actual yields of Record arranged in order of magnitude were: 4.3, 11.0, 12.0, 16.4, 26.0 and 28.0 cwt. per acre. The chart brings out clearly the very marked relative superiority of Radnorshire Sprig and Black Bell III at the centres where Record falls in yield below 12 cwt. of grain per acre.¹

In testing at the Welsh Plant Breeding Station the yields of bred strains belonging to the different agronomic groups of oats some additional evidence has recently been obtained which shows the same trend of behaviour.

¹ For a more detailed account of these earlier investigations the reader is referred to *Bull. Welsh Pl. Breed. Sta.* Series C, No. 3 (1923).

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The data are as follows:

	Grain cwt. per acre	Straw cwt. per acre
<i>Wood Field, 1932.</i>		
(1) Plots:		
Radnorshire Sprig	13.9	14.9
Ceirch Llwyd	15.5	28.2
<i>Lower Ridge Field, 1930.</i>		
(2) Rod-row trials:		
Radnorshire Sprig	21.5	33.0
Ceirch Llwyd	15.7	36.5
Record	18.1	36.1
<i>Gorse Field, 1932.</i>		
(3) Rod-row trials:		
Series C. 223		
Radnorshire Sprig	30.5	34.7
Thousand Dollar	32.2	41.4
Series C. 234		
Thousand Dollar	33.9	42.9
Record	36.3	44.3

Comparison (1) is between five-times replicated 1/100th acre plots on adjacent areas of land in the same field. Comparisons (2) and (3) are averages of six- to eight-times replicated "protected" rod-row trials.

The figures are incomplete in so far as that Record is not included in all the comparisons, but marked relative differences of yield and differences of ranking are nevertheless apparent. In this case, however, they appear at a slightly higher level of productivity than those shown in Chart 1.

At approximately the 15-cwt. level for Radnorshire Sprig, it will be observed that Ceirch Llwyd has given slightly higher yields of grain and a very marked increase of straw over Radnorshire Sprig.

At approximately the 20-cwt. level, however, the situation is changed and Radnorshire Sprig surpasses in grain both Record and Ceirch Llwyd and gives an improved yield of straw, while at a productivity level of approximately 30 cwt. Record takes first place to Radnorshire Sprig in both straw and grain.

Although the above trials were not specially laid down to test the points at issue, the evidence they provide is admissible and agrees in showing the same significant changes in the relative behaviour of varieties according to the levels of productivity at which they are compared.

The practical implications of the earlier investigations have been discussed by Stapledon (6) in their bearing upon the choice by the farmer of the most suitable variety to grow. In the same paper he has given a classification of the existing and most useful varieties suited to the varied

conditions prevailing in Wales. These are arranged according to their adaptability to soils of different productive capacity, and the varieties are divided into five categories according to their ability to do well at prescribed levels of crop expectancy.

These examples of the differential response of varieties are fundamentally important to the problem of yield testing and especially so in relation to the oat crop which is commonly grown over a wide and diversified range of conditions.

This phenomenon of the change in the ranking of varieties when grown under conditions of high, medium or low cropping capacity is, however, not confined to the oat crop. It has been observed by Mooers (4) in maize and by Beaven (1) in comparing the varieties Plumage and Archer in barley.

In endeavouring to trace additional evidence of the differential response of oat varieties the writer has examined the published reports of a number of oat trials conducted in the counties within the Aberystwyth area from 1920 onwards (7, 8, 9, 10, 11, 12, 13, 14). A summary of the varieties grown and their average yields of grain and straw is given in Table I. These data relate mainly to upland or semi-upland conditions.

Table I.
County trials in the Aberystwyth area 1920-7.

	Average yields	
	Grain cwt. per acre	Straw cwt. per acre
Record	14.9 (29)*	28.8 (15)*
Victory	15.0 (17)	27.5 (6)
Golden Rain	19.1 (16)	33.0 (9)
Abundance	13.7 (14)	31.1 (5)
Supreme	13.6 (12)	33.0 (2)
Yielder	11.3 (12)	25.9 (7)
Crown	16.6 (5)	22.2 (2)
Orion	16.1 (3)	22.7 (3)
Radnorshire Sprig	15.3 (24)	23.2 (15)
Scotch Potato	12.6 (24)	28.1 (15)
Black Tartarian	14.8 (12)	26.0 (7)
Black Bell III	18.4 (15)	32.1 (12)
Englebrekt	11.3 (1)	—
Ceirch-du-bach	8.1 (12)	24.2 (7)
Ceirch Llwyd	9.0 (4)	19.2 (4)
Castleton	10.0 (2)	—
Odal	14.6 (3)	34.8 (3)
Banner	12.8 (1)	16.0 (1)
Goldfinder	19.5 (1)	24.8 (1)
Bountiful	8.4 (5)	22.6 (2)
Varietal average	13.7	26.4

* The figures in brackets indicate the number of centres contributing to the figure for average yields.

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It will be seen from the table that a large number of varieties has been tested, but some only to a limited extent. Owing to the varying number of centres contributing to the varietal averages and the wide range of yields which are manifest from centre to centre, direct comparisons between variety and variety would possibly in many cases be misleading. Two varieties, however, deserve attention in a general way, namely Golden Rain and Black Bell III. These have been included at a large number of centres and on the average have given very good results. They are varieties which have been bred at Svälöf for soils of medium and medium-low productivity respectively.

In order to seek information on the behaviour of type varieties, the trials at all centres have been examined from this point of view. Unfortunately only an insignificant number were composed of varieties representative of all three groups, but there were eight trials in each of which were included varieties appropriate to soils of high and medium productivity respectively.¹ These have been separately considered and an analysis of the yield figures is given in Table II. Three varieties are considered, namely Record as representative of the Grade I group and Radnorshire Sprig and Black Bell III as examples of the Grade II group. When all centres, of which there were eight, are considered together (see Table II, comparison (a)) such advantage in grain yield as the figures show is slightly in favour of the Grade II varieties. In straw yields, however, Radnorshire Sprig is definitely below Record, while Black Bell III is practically equal to the latter variety.

If we exclude all centres (comparison (b)) at which Record exceeded 20 cwt. in yield of grain (of which there were three, comparison (d)) the advantage in grain yield is seen to swing more markedly in favour of the Grade II varieties (113 and 115), with Radnorshire Sprig now very nearly equalling Record in yield of straw, while Black Bell III straw weights are 9 per cent. higher than Record.

To proceed to a still lower level of productivity by excluding all centres where Record exceeded 12 cwt. in yield of grain (comparison (c)),

¹ For the convenience of the reader, a grouping of oat varieties as now adopted, in conformity with their suitability for conditions of high, medium or low productivity (which may be conveniently referred to as Grade I, Grade II and Grade III groups respectively) is given below. The grouping is similar to that employed by Stapledon⁽⁶⁾ except that the varieties are for present purposes arranged in three groups instead of five:

Land of high productive capacity (=Grade I): Record, Victory, Star, Supreme, Golden Rain II, Marvellous, Eagle, Elder.

Land of medium productive capacity (=Grade II): Radnorshire Sprig, Black Bell III, Black Tartarian, Scotch Potato, Englebrekt.

Land of low productive capacity (=Grade III): Ceirch-du-bach, Ceirch Llwyd.

Radnorshire Sprig is seen to maintain its relative position in respect of grain, coupled with a slight relative improvement in straw. Black Bell III, however, while nearly maintaining its former level in straw yield, wins by a substantial margin in weight of grain.

Table II.

A comparison of the yields of varieties representative of the Grade I and Grade II groups of oats (see county data, 1920-7).

	Grain cwt. per acre	Straw cwt. per acre
<i>Comparison (a).</i> All centres at which Record (Grade I) and Radnorshire Sprig and Black Bell III (Grade II) were grown.		
Record	16.8 (100)	27.7 (100)
Radnorshire Sprig	17.5 (104)	23.8 (86)
Black Bell III	17.7 (105)	28.3 (102)
<i>Comparison (b).</i> Where Record gave less than 20 cwt. grain per acre (5 centres).		
Record	13.1 (100)	19.3 (100)
Radnorshire Sprig	14.8 (113)	19.0 (98)
Black Bell III	15.1 (115)	21.1 (109)
<i>Comparison (c).</i> Where Record gave less than 12 cwt. grain per acre (2 centres).		
Record	9.7 (100)	15.5 (100)
Radnorshire Sprig	11.0 (113)	16.3 (105)
Black Bell III	12.6 (130)	16.7 (107)
<i>Comparison (d).</i> Where Record gave more than 20 cwt. grain per acre (3 centres).		
Record	23.0 (100)	36.1 (100)
Radnorshire Sprig	21.9 (95)	28.6 (79)
Black Bell III	22.1 (96)	35.5 (98)

When, on the other hand, we take the three centres at which Record exceeded 20 cwt. in grain production, and consider these together (comparison (d)), we find the position reversed, being now wholly in favour of the Grade I variety Record—the Grade II varieties falling short in yields of both grain and straw. It would have been interesting in all these instances to have been able to compare kernel weights per acre.

It must be made quite clear that the number of county trials here reviewed is small, and the data belong mainly to single-plot trials at the respective centres. The data are therefore very inadequate, but nevertheless they point in the same general direction, as do the previous studies in this kind of investigation. They show distinctly the changes in relative yields at the different levels of productivity and also a general tendency of the Grade II varieties to exhibit an accentuated increase in relative superiority with each fall in the level of productivity. Both the county yield trial figures and the Welsh Plant Breeding Station data seem to agree in indicating that the change-over in relative superiority of grain yield between Grade I and II varieties occurs at some point between the 15 and 20 cwt. level of productivity of the standard variety

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Record. A more precise determination is needed, but more extensive investigation is necessary before such can be made.

OAT YIELDS IN THE PRINCIPALITY.

If we examine the estimated figures of yield as contained in the agricultural statistics on average grain yields as issued by the Ministry of Agriculture (18) we find the average yield of oats in Wales during the ten-year period 1921–30 to be 11·7 cwt. per acre—the comparable figure for England being 15·0 cwt. This figure for the Principality seems low compared with data from actual field trials. Thus the varietal average for the county data presented in Table I is 13·7 cwt., and these trials relate mainly to upland and semi-upland conditions.

Of trials conducted at higher levels of productivity, the extensive scheme of trials with spring sown oats designed and organised by the National Institute of Agricultural Botany furnish useful data. These trials have been in operation during the last four seasons, and a number of selected Grade I varieties have been grown at a number of centres in England and Wales. In an analysis of the results of the 1931 trials discussed by Parker (5) the average yield of the three leading varieties, Golden Rain II, Victory and Marvellous, obtained at twenty-four centres in Wales was 20·9 cwt. per acre, which is identical with the average of 20·9 for these three varieties at all centres at which they were tested in England and Wales.

An almost uninterrupted series of oat trials has been conducted at the farm of the University College of North Wales from 1911 up to the present time. A summary of the grain yields (converted into cwt. per acre) taken from the published reports for the years 1911–32 (15, 16, 17) is given below:

	cwt. per acre
Record (home-grown and new seed)	30·2 (14 and 8)*
Victory	31·2 (12)
Golden Rain	24·4 (11)
Scotch Potato	24·2 (1)
Black Tartarian	26·4 (12)
Black Bell III	30·1 (5)
Englebrekt	30·1 (4)
Golden Rain II	34·0 (3)
Average	28·8

* The figures in brackets show the number of seasons in which the respective varieties were grown and of which the preceding figure is the average.

Average grain yields for varieties tested at the University College of Wales, Aberystwyth for the three seasons 1931–3¹ are as follows:

¹ Unpublished data by the courtesy of Prof. J. Jones Griffith.

	cwt. per acre
Golden Rain II	27.7
Victory	27.3
Marvellous	25.0
Record	24.8
Star (1933 only)	27.0
Eagle (1931 only)	26.0
Average	26.3

If we calculate the mean of the average yield in these three sets of data, a yield figure of 25.3 cwt. per acre is obtained which is slightly more than double the official estimate for the Principality. Having in mind these data and the official estimate itself, one thing seems clear, namely, the marked disparity which exists between the two. In partial explanation of this, there is the possibility that in the official estimates inadequate allowance is being made in respect of roads, cart tracks, fences and inaccessible corners which make up the gross area of the field as shown by the Ordnance map and which do not contribute to the actual yield of the crop. The experimental data on the other hand are based on net acreage. In certain instances too the area of experiment, which has to be chosen for its uniformity, is perhaps slightly better than the average in productivity compared with the surrounding part of the field. This, however, is not always so. There is, moreover, the possibility that the data obtained by experiment lack a sufficient proportion of tests at the lower and lowest levels of productivity.

After consideration of these possible causes it still remains difficult to avoid the conclusion that the estimated figure is low as a representation of the actual average yields.

In the light of evidence submitted in the earlier part of this paper, knowledge of the actual level of productivity is important in relation to the problem of the choice of the right variety to grow, and if the yield level even approximates to that of the estimated figure, we have in Wales a vast area of land on which it would be best to grow Grade II and even Grade III varieties and which up to the present is not being proportionately represented in our variety testing experiments. It is believed, however, that an extended system of type trials should give useful information upon the general situation in this respect. Such trials would not only give information on the relative order of merit of varieties as between members of the Grades I, II and III groups at different levels of fertility, but by being grown over a wide and representative range of fertility conditions, the actual as distinct from the relative yields should be of value in computing a figure for the average output of grain per acre in the Principality.

GENERAL CONSIDERATIONS.

Several practical issues arise in connection with these observations, the answers to which cannot be given until further data have been obtained. There is in the first place the very important issue from the practical and advisory point of view, which has already been commented upon, of the level of productivity at which a farmer should change over from a given variety of Grade I to a member of the Grade II or Grade III groups and *vice versa*. On this issue the data hitherto assembled are not complete and critical enough to permit of a decision being made, although there are very definite indications that at levels below 15 or 18 cwt. the Grade I varieties are generally outyielded by some member of the Grade II or Grade III groups.

There is also the effect of seasonal climatic conditions upon the actual levels of yield. Thus, for example, it is necessary to enquire whether the relative order of merit of a given set of varieties when grown at a centre of low fertility in a season highly favourable to growth is the same as that at a centre of known higher fertility in a season unfavourable to growth—the crops under the conditions stated assumedly showing similar actual yields. In the figures for yield so far investigated, the ranking of the varieties has been considered wholly on the basis of the absolute yields of a standard variety from centre to centre, without regard to seasonal influences thereon. A study of the latter is undoubtedly desirable, but the meagreness of the data available renders a correlated study of these influences impossible at the present time. The seasonal factor adds an element of complexity to the results inasmuch as low fertility and low productivity do not go together, for, in occasional seasons, the magnitude of the seasonal influence may be sufficient to raise the level of productivity from that under which a Grade III variety does best to a level where a Grade I variety takes the lead.

In order to obtain the maximum crop, especially on land of medium and medium to low fertility, the grower has not only to make an estimate of the cropping capacity of the field and form some opinion of its probable yield in relation to possible seasonal conditions, but must, in relation thereto, choose the right variety. In doing so it is important not to overestimate the cropping capacity and to select if need be a Grade II or Grade III variety, for a full crop of a second grade variety as pointed out by Jones⁽³⁾ is likely to be of more value to the grower who feeds the produce to his stock than a 75 per cent. crop of a first grade variety.

Of no little importance in relation to the usefulness and value of a variety is its ability to form well-filled grain under adverse ripening conditions. This is especially true as regards the suitability of a variety for upland areas. On upland farms conditions for good grain formation are frequently unsatisfactory, and the hill farmer in his choice of a variety must guard himself against employing one which fails to give grain good enough in an average season to be utilised, if need be, as seed in the following year.

Evidence on the behaviour of varieties in this respect could very well be obtained by carefully harvesting seed from trial plots and using this for laying down repeated trials in one, two and more seasons.

The introduction of seed treatments, such as some of the new mercuric dust compounds now on the market, into farm practice, brings with it a fresh need for further agronomic investigation. If better and denser braids can be established by seed disinfection (and under certain conditions not yet fully understood there are indications that this may be so), the employment of varieties belonging to a higher grade may be possible in areas where now a lower grade variety is being grown. Quite frequently on hill farms the getting of a good crop of a heavy-grained variety is only seldom possible owing partly at least to a failure to develop a sufficiently dense growth in the early part of the growing season. Whether or not this can be overcome by treating the seed with a prepared chemical dust is a matter deserving attention. Owing, however, to the inconsistent results obtained under different conditions by the use of the various chemical preparations now on the market¹ more detailed information is wanted on the positive effects of seed treatment and on the particular and precise conditions under which they act to the best advantage. It appears at least from certain investigations that differences between treated and untreated seed samples are most marked where the climatic conditions immediately subsequent to sowing are unfavourable to growth.

The possible usefulness of seed treatments in the direction indicated cannot, however, be taken advantage of with any degree of regular and confident success until more consistent results can be obtained with the materials and methods now employed.

¹ Unpublished data from current investigation by Mr D. W. Davies, Advisory Mycologist, and the writer.

GENERAL DISTRIBUTION OF VARIETIES.

Of much interest and value in connection with the ecological adaptation of oat varieties would be the construction of a survey map showing the geographical distribution of the more widely grown varieties. This, however, would be a great and difficult task. Some indication of the extent to which different varieties are grown in Wales may be obtained, however, from the quantities of "seed" oats of different varieties sold over a number of seasons in different parts of the Principality. An initial enquiry of this kind has been made for the season 1933.

Through the assistance of the Organiser of the Welsh Agricultural Organisation Society, Ltd., and the Managers of the Welsh Agricultural Co-operative Societies, data have been collected relating to sales at twenty-nine centres in the counties of Anglesey, Brecon, Cardigan, Caernarvon, Carmarthen, Denbigh, Flint, Glamorgan, Merioneth, Pembroke and Radnor which on a liberal basis of a seed rate of 2 cwt. per acre would represent approximately 8 per cent. of the whole area sown with oats in these counties in an average season.¹ Proportionately few returns, however, were obtained from Anglesey, Denbigh, Flint, Montgomery and Merioneth, mainly areas, excepting Merioneth, in which Grade II and III varieties are rather less widely grown. The data, therefore, are possibly not truly representative of conditions in the Principality as a whole.

The varieties sold and their proportions on a percentage basis are given below:

Grade I varieties:	Victory	31.44	} = 53.31 %
	Abundance	7.83	
	Golden Rain and Goldfinder	5.36	
	Supreme	4.17	
	Record	1.82	
	Superb	1.51	
	Yielder	1.18	
Grade II varieties:	Scotch Potato	16.09	} = 40.56 %
	Castleton	9.54	
	Radnorshire Sprig	8.22	
	Black Tartarian	6.71	
Grade III varieties:	Ceirch-du-bach	3.53	} = 6.12 %
	Ceirch Llwyd	2.59	

One striking feature to be observed is that in the area served by these centres in this particular season 46 per cent. of the "seed" sold is

¹ The writer is indebted to Mr T. Lewis, B.Sc., M.S. (formerly Organiser of the W.A.O.S., now Regional Officer, Milk Marketing Board), and Mr Hugh James, B.A., Secretary, W.A.O.S., for organising the collection of these data and to the managers of the respective Societies for the readiness with which they furnished the information required.

of Grades II and III varieties. Another is the pre-eminence of Victory in the Grade I group. Scotch Potato and its allied variety also form a high proportion of the supplies in the Grade II group. We, however, know by experiment that Victory is, on the average, the highest yielding of the varieties listed in the Grade I group, and it is interesting to observe that, in the comparatively short space of time this variety has been on the market in this country, it has so rapidly and markedly gained recognition by the farmer. The varieties in the Grade II group, however, are of longer standing, but we can likewise presume that, by the processes of trial and observation which have brought Victory to the fore in the Grade I group, the farmer after many years of trial and observation has singled out these Grade II varieties as being the best for the districts in which they are now being still so very widely grown. On the basis of the known superiority of these varieties at the lower levels of productivity the continued demand for them is not altogether unexpected. Its magnitude, however, is perhaps a little surprising.

If a classification is made of the data supplied by the twenty-nine Societies here considered into (a) Societies which sell more Grade I varieties than Grades II and III taken together, and (b) conversely, a very marked cleavage in the proportions of these grades sold is manifest:

(a) *Societies selling more Grade I than Grades II and III varieties:* Ammanford, Arthog, Carmarthen and District, Clynog Fawr, Emlyn, Eifionydd, Foel, Llanelly, Llangyfelach, Llanbedr, Dyffryn, Mid-Glamorgan, Pembroke and District, Penybont, Swansea and District, Vale of Clwyd. Grade I, 70·3 per cent.; Grades II and III, 29·7 per cent.

(b) *Societies selling equal or less amounts of Grade I than Grades II and III varieties:* Aberystwyth, Blaenpennal, Crymmych, Corwen, Dolgelly, Edeyrnion, Llandyssul, Llanybyther, Llandovery, Llangadock, Pumpsaint, Vale of Aeron, West Brecon, Yspsyty Ifan. Grade I, 29·3 per cent.; Grades II and III, 70·7 per cent.

This classification of the Societies automatically separates them into those mainly serving the lowland districts and those mainly serving the upland areas, and the respective difference in the relative quantities of the grades sold is very marked. If allowance is made for the slightly lesser weights of Grades II and III seed than Grade I required to sow equal areas, the division made above represents approximately equal areas, the actual weights of seed sold being in the ratio of (a) 53 to (b) 47. This makes the significance of the proportions all the more important.

The big demand for Grades II and III varieties, and especially the former, still prevailing in certain districts, coupled with the official esti-

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mated average yields of oats in Wales, deserve close attention in relation to variety testing. They seem to point to a necessity for more extended trials and especially for trials at the lower levels of productivity.

In addition to the more usual kind of trial comprised of varieties belonging to the same grade or group, more detailed information on the ecological adaptation of varieties is much required. This can only be obtained from trials constituted on a type basis and conducted over a wide and varied area. Such trials it would appear should be the logical forerunner of the former, for not until we have more conclusive evidence of the levels of demarcation between soils of high, medium and low productivity can we intelligently employ the right group in the right situation.

It is believed that a unified and planned system of type trials conducted over the whole of the Welsh counties should furnish the much needed information.

OTHER FACTORS OF PRACTICAL IMPORTANCE IN VARIETY TRIALS.

Although it is not intended to enter into any detailed discussion of the many factors contributing to the value of an oat variety from both the farmer's and purchaser's points of view, some reference must be made to the problems of the priority in economic importance which should be given in variety trials to such major oat characters as:

- (1) Straw stiffness.
- (2) Yield (including proportion of "head corn").
- (3) Grain size, shape and quality.
- (4) Resistance to disease.
- (5) Time of maturity.

The issue here lies in the fact that varieties which may not show any significant superiority in yield may possess other attributes of practical and economic value, some one and others another.

Most of these, however, are features which concern more closely the members of the Grade I group inasmuch as they are grown for a wider diversity of purposes than are members of the other groups.

Some of the recently introduced new varieties belonging to the Grade I group differ appreciably in their hereditary make-up in respect of one or more of these main characters. Taking recent and even past introductions as a whole, the individual varieties excel only in one, two or perhaps three of these main features. It is possible, and very highly probable, that the introductions of the near future will also fall

short of an "ideal oat" in at least some one or other of the already mentioned desirable attributes, and be perhaps more highly developed and more specialised in one or more features. Even disregarding all this, there is still the probability that growers themselves may hold quite different views as to the values to be set upon any one or more of these main attributes, being influenced by the nature of the district, the degree of fertility of the soil with which they have to deal, or the system of farming practised. These considerations, undoubtedly, predetermine the relative importance of these varietal characters from place to place.

If the soil is of an exceptionally high degree of fertility, straw stiffness is fundamentally essential. Moreover, if a grass seeds mixture is sown along with the crop, the value of a variety which does not lodge is still more greatly enhanced even if its grain yield is slightly inferior to that of the best grain-yielding variety. In a late district a certain degree of earliness is a necessity. In growing oats for milling, quality demands first place, and so on.

With all these differences and divergences to consider, a need is felt for some agreement of opinion as to how in our system of testing varieties a proper and satisfactory evaluation of these different factors should be carried out. How much importance should be placed on yield? How much and what value should be given to straw stiffness and how much deduction should be made, or handicap given, for differences in time of maturity? To what extent should any set-off be made in respect of deficiencies in yield of grain or straw in a variety which otherwise is outstanding in earliness of maturity, straw stiffness and grain quality? These are critical issues, and their correct and standardised evaluation is important not only in the testing of varieties but also to the plant breeder.

There seem to be two ways of approaching the problem: firstly, we may assign to these main attributes certain more or less definite numerical values and assess the variety on the total score so obtained; or, secondly, we may decide that, for certain conditions or certain requirements, particular characters should take a certain priority of position. In this latter case separate standards of evaluation would have to be adopted in variety trials. Such values would depend upon the requirements of the area.

In this latter case specialised varieties would possibly receive more individualistic consideration, and the necessity for slightly different "models" of varieties, even within the limits of the grade or group, would be an expected and accepted state of affairs.

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Some of us would perhaps agree that the order of priority of the main attributes should be in the order listed above. Others perhaps would place yield first, and others again some other character. We may, however, perhaps agree that one solution is to have a set of varieties, each differently constituted in relation to these characters, from which to choose the particular kind of variety we require. If this is so, then our trials should be arranged and judged accordingly. This may mean a multiplicity of varieties, but providing they are adequately tested and appropriately described and categorised, it may, in the long run, prove to be the least disadvantageous course to pursue.

SUMMARY.

In former investigations certain varieties of oats were found to vary in order of yield, depending upon whether they were compared at centres of high or of low productivity.

New data are presented in which the contrasting agronomic types Ceirch Llwyd, Radnorshire Sprig and Record were studied. These figures confirm the earlier investigations. The superiority of the higher tillering varieties was, however, maintained up to a higher level of yield than in the former studies, while that of Ceirch Llwyd at the lowest levels of yield was very marked.

Yield data for a large number of trials conducted in certain counties in Wales are summarised. An analysis of these shows the same characteristic changes. Here Record gave a superiority of yield at the 23.0 cwt. level of 4.5 per cent. over the average yields of Radnorshire Sprig and Black Bell III. At the level of 16.8 cwt. it was itself surpassed by an equal margin by these varieties, the superiority of which increased to 11.4 per cent. at the 13.1 cwt. level and to 21.5 per cent. when the yield of Record was 9.7 cwt. In these data the accentuated superiority of Black Bell III over Record with each successive fall in the level of productivity was very pronounced.

These data and others from soils of higher fertility are compared with the official estimated average yield of oats in Wales of 11.7 cwt. per acre. The lowness of the latter figure is discussed and considered in relation to the general problem of yield testing, the placement of varieties in order of merit of yield and other practical issues.

A grouping is given of the more commonly grown varieties of oats into three grades or groups in conformity with their suitability for soils of high, medium or low productivity. More data are needed in order to define more closely the limitations of the groups. The general insuffi-

ciency of the data in this respect emphasises the necessity for a planned scheme of type trials (as distinct from the generally employed group testing of varieties) as a means of obtaining more precise information. In connection with trials at the lower levels of yield, their repetition with once, twice or even thrice grown seed is desired.

The treatment of seed with mercuric dust compounds is briefly discussed in relation to oat growing on upland farms.

A single year survey of the kind and proportion of oat varieties grown in Wales based upon certain sales of "seed" oats in 1933 showed that approximately 43 per cent. of the seed sold belonged to Grades II and III groups—mainly Grade II.

Observations are made on the practical importance of, and priority values to be given to, straw stiffness, disease resistance, grain quality and time of maturity in relation to yield, and on the need for some agreed basis of evaluation.

It is suggested that in consequence of the differential response of varieties and of the several factors which predetermine the value of a variety from place to place, increased attention should be given to testing of varieties in relation to locality and local requirements.

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INCIDENCE OF TAKE-ALL ON WHEAT AND BARLEY ON EXPERIMENTAL PLOTS AT WOBURN

By MARY D. GLYNNE.

(*Department of Plant Pathology, Rothamsted Experimental
Station, Harpenden, Herts.*)

(With 1 Text-figure.)

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INTRODUCTION.

A SEVERE attack of take-all, caused by the fungus *Ophiobolus graminis* Sacc., was found in 1931 on certain plots of the continuous wheat experiment on Stackyard field at the Woburn Experimental Station. The incidence of the disease varied so much in differently manured plots that a detailed survey seemed desirable. This was carried out in 1931 and in the two succeeding years, when the numbers of clean and diseased plants and their yields were determined for ten random samples taken from each plot just before harvest. Take-all was also found, but less abundantly, on the continuous barley plots under similar manurial treatment and adjacent to the wheat. These were surveyed in 1932 and 1933.

METHODS.

Ten spots were selected for sampling by the process of randomisation. Each sample consisted of all the plants in two half-metre lengths separated by a metre and taken from adjacent rows. They were uprooted carefully and later each plant was examined, tearing away the sheath and lower leaves to expose the characteristic black "plate" mycelium. Perithecia were often present. The straw and grain of diseased and of clean plants in each sample were weighed separately.

CONTINUOUS WHEAT AND BARLEY EXPERIMENTS,
STACKYARD FIELD.*History and arrangement of plots.*

Experiments on the continuous growing of wheat and barley on the same land under various manurial treatments were laid out in adjacent parts of Stackyard field in 1877, and these crops were grown with manure applied annually till 1926, when the land had become so badly infested with weeds that it was left fallow for the two years 1927 and 1928. Wheat and barley were cropped each year from 1929 to 1933, and in 1934 the land was again left fallow. No manures were applied to wheat after those put on the 1925-6 crop, and none to barley except in 1931 and 1932 when manures were applied to eight¹ of the twenty-six plots. "Square Heads Master" wheat was grown in 1931 and "Red Standard" in 1932 and 1933. Alternate rows of the varieties "Plumage" and "Archer" barley were grown in 1932, and in 1933 only the one variety "Plumage Archer". There is no record of disease in the wheat or barley plots before 1931 when they were first examined in detail from a pathological viewpoint.

The pH determinations were made by the Chemistry Department. The effects of the fertilisers and lime on the soil reactions and exchangeable base contents have been described by Crowther and Basu⁽¹⁾ and by Crowther⁽²⁾.

*Incidence of take-all in relation to conditions.**(a) Manurial treatment.*

As manures were last applied to all the wheat and most of the barley plots in the autumn of 1925 it is mainly the effect of manurial residues that are considered here. The incidence of take-all is expressed as percentage plants diseased. Tillers were also counted and, as the plants were mostly in a starved condition, tillering was low, an average of 1.29,

¹ For details of manuring see Note 1, following Table I.

1.18 and 1.25 tillers per clean plant and 1.20, 1.13 and 1.13 tillers per diseased plant being found in the three successive years.

In wheat the unmanured plots, and one which had received minerals only, showed a fairly heavy infection, varying from 13.5 to 43.2 per cent. Four plots which had received ammonium sulphate but no lime, and four others similarly treated with the addition of 1 or 2 tons of lime showed little or no disease. Plot 2BB, which in addition to ammonium sulphate had received 4 tons of lime, and plot 5B, which had mineral manure annually and 1 ton of lime, had a high percentage of infected plants. Plots which had received nitrate of soda all showed a considerable amount of take-all by 1933. There was very little disease on plot 10B which had received rape dust, and a high percentage on plot 11B to which farmyard manure had been applied. The maximum percentage of plants infected by take-all in any plot was 52.5.

In barley, infection was on the whole very much less than on corresponding wheat plots, the maximum being 16.2 per cent. As with wheat there was little or no infection on the plots which had received ammonium sulphate alone, but very little crop was obtained as barley is more sensitive to acidity than wheat. Plots 2AA, 2B, 8AA and 8BB with ammonium sulphate had received more lime and so had a higher *pH* than corresponding wheat plots. They also had more take-all. All plots which had been treated with sodium nitrate and plot 11B with farmyard manure showed some degree of infection, while plot 10B, which had received rape dust, was very slightly infected. Manuring of plots 8A, 8B, 8AA, 8BB, 9A, 9B, and 10A and 11A in 1931 and 1932 showed no obvious effect on the incidence of take-all in 1932 and 1933.

(b) *Soil pH.*

The relation of soil *pH* to percentage take-all is shown in Table II. Little or no disease appeared in either wheat or barley when the *pH* was 5.0 or less. A little appeared at *pH* 5.1, and at higher *pH* values take-all was more plentiful, all the wheat plots except plot 10B showing considerable infection, at least 16 per cent. by 1933, and all the barley plots showing some infection. Wide differences in the amount of take-all were found in plots with the same *pH*, but when the *pH* values were taken in pairs and the percentage infection averaged a general tendency was seen for infection to rise with increase in *pH* in the wheat plots, but this was not apparent in the barley. The highest *pH* values were 5.9 for wheat and 6.4 for barley plots, both on the acid side of neutrality, so no indication is obtained regarding the behaviour of take-all in alkaline soil.

Table I. *Percentage take-all on wheat and barley with pH values of soil.*

Plot No.		Wheat					Barley						
		Lime added (tons per acre)	pH of plot 1932	Percentage plants with take-all			Lime added (tons per acre)	pH of plot 1932	Percentage plants with take-all				
				Standard error	1932	Standard error			1933	1932	1933		
*Manures applied per acre annually to 1926. Two years fallow 1926-8, added No manures applied afterwards except to certain barley plots, in 1931 and 1932 (see Note 1)													
No nitrogen:													
1	Unmanured	—	5.6	23.4	±6.7	31.8	±6.9	35.2	±6.1	—	5.4	8.0	8.1
7	Unmanured	—	5.5	13.5	±6.7	20.6	±7.5	43.2	±9.1	—	5.6	13.8	8.4
4 (A)	Minerals (superphosphate and sulphate of potash)	—	5.8	28.7	±7.7	36.0	±7.5	32.6	±5.5	—	5.4	6.8	10.9
(B)	"	—	—	—	—	—	—	—	—	1.0	5.7	13.1	9.2
Ammonium sulphate:													
2A	0.184 cwt. N	—	4.7	0	—	0	—	0	—	—	4.4	0	1.8
5A	0.184 cwt. N + minerals	—	5.1	0	—	0	—	2.2	—	—	4.7	0	0
8A	0.368 cwt. N in alternate years	—	4.8	0	—	0	—	0	—	—	4.7	0	0
8B	+ minerals every year	—	4.8	0	—	0	—	0	—	—	5.0	0	1.0
Ammonium sulphate + lime:													
2AA	0.184 cwt. N as 2A	1.0	4.9	0	—	0.3	—	0	—	1.5	5.1	2.7	5.7
2B	0.184 cwt. N as 2A	2.0	5.0	0	—	0.4	—	0	—	4.0	5.8	5.1	3.7
2BB	0.184 cwt. N as 2A	4.0	5.3	14.5	±3.4	35.0	±6.5	23.0	±6.3	4.0	5.9	3.9	2.7
5AA	0.184 cwt. N + minerals as 5A	—	—	—	—	—	—	—	—	2.0	5.0	0	3.4
5B	0.184 cwt. N + minerals as 5A	1.0	5.6	43.8	±10.8	44.7	±9.4	16.0	±5.3	4.0	5.9	8.9	15.1
8AA	0.368 cwt. N in alternate years	1.0	5.0	0.3	—	0	—	0	—	4.0	5.8	9.0	9.6
8BB	+ minerals every year, as 8A and 8B	1.0	5.1	0	—	2.3	±0.8	8.4	±5.4	4.0	5.9	3.6	11.5
Sodium nitrate:													
3A	0.368 cwt. N	—	5.8	30.8	±8.3	35.0	±7.7	28.9	±8.7	—	5.9	1.8	5.8
3B	0.184 cwt. N	—	5.6	20.0	±5.0	40.7	±5.4	29.5	±4.5	—	5.6	4.9	12.2
6	0.184 cwt. N + minerals	—	5.8	17.9	±2.3	29.8	±3.2	39.8	±7.3	—	5.8	9.2	13.0
9A	0.368 cwt. N in alternate years	—	5.8	23.1	±4.1	52.5	±5.4	42.7	±6.0	—	5.7	5.5	6.3
9B	+ minerals every year	—	5.7	23.6	±6.3	24.5	±7.5	28.5	±5.5	—	5.8	1.8	1.0
10A	0.184 cwt. N and superphosphate (after dung and no manure)	—	5.6	1.9	±1.9	3.8	±1.8	34.5	±8.0	—	5.6	6.0	2.3
11A	0.184 cwt. N + potassium sulphate (after dung and no manure)	—	5.8	9.6	±2.7	31.8	±5.8	38.9	±5.9	—	5.6	13.5	1.3
Sodium nitrate and lime:													
3AA	0.368 cwt. N	—	—	—	—	—	—	—	—	2.0	6.4	1.5	8.2
3BB	0.184 cwt. N	—	—	—	—	—	—	—	—	2.0	6.3	1.3	5.0
Organic manure:													
10B	0.184 cwt. N as rape dust	—	5.4	0	—	0	—	2.4	—	—	5.1	0	1.2
11B	0.75 cwt. N as farmyard manure	—	5.9	36.1	±5.6	43.1	±4.5	33.2	±6.3	—	5.8	16.2	10.1

* For details of manurial treatment see *Rep. Rothamst. exp. Sta. (1927-8)* (3).

The wheat plot 10B which had received rape dust was exceptional in that though it had a pH of 5.4 little infection appeared. The corresponding barley plot also had little take-all, but its pH was only 5.1. The possibility suggests itself that rape dust may have a specific effect on the fungus, and it has been observed in Germany that a preceding crop of *Brassica* has an inhibitive effect on take-all. In the absence of further evidence, however, the suggestion can only be regarded as a very tentative one.

(c) *Yield.*

No clear relation appears between yield and percentage take-all. Of the eight plots giving the highest average yield for the three years 1931-3 four were practically free from the disease and four rather badly affected. For instance, plot 5A, which received ammonium sulphate and minerals and had a *pH* of 5.1, was practically free from disease and produced crops equal to and, in recent years, superior to any of the other plots.

Variation in infection 1931-3.

The variation in percentage infection of wheat on each affected plot is shown graphically in Fig. 1. From 1931 to 1932 take-all increased in all plots which showed infection in 1931 and in one clean plot. All plots

Notes to Table I.

(1) Manuring of barley in 1931 and 1932:

Plots	Quantity per acre
1-7	Unmanured
8A, 8B, 8AA, 8BB	3 cwt. superphosphate, 1½ cwt. sulphate of potash, 1½ cwt. sulphate of ammonia
9A, 9B	3 cwt. superphosphate, 1½ cwt. sulphate of potash, 2.28 cwt. nitrate of soda
10A	3 cwt. superphosphate, 2.36 cwt. nitrate of soda
10B	Unmanured
11A	1½ cwt. sulphate of potash, 2.36 cwt. nitrate of soda
11B	Unmanured

(2) Total amounts of N, P₂O₅, K₂O applied in tons per acre:

	1877-1906	1907-26	Total 1877-1926
N (to plots 2, 3 B, 5, 6, 8, 9)	0.55	0.18	0.73
P ₂ O ₅ (to plots 4, 5, 6, 8, 9)	0.84	0.48	1.32
K ₂ O (to plots 4, 5, 6, 8, 9)	1.34	0.25	1.59

(3) Arrangement of plots:

Wheat					Barley									
1	2AA	2BB	3A	3B					3BB	3AA	2BB	2AA	1	
	2A	2B			10A	10B	10A	10B	3B	3A	2B	2A		
4	5A	5B	6						6		5B	5A	4B	
					11A	11B	11A	11B				5AA	4A	
7	8BB	8AA	9B	9A					9B	9A	8BB	8AA	7	
	8B	8A									8B	8A		

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which in 1932 showed infection but had less than 35 per cent. plants infected, as well as two clean plots, showed increased infection in 1933. Seven plots had 35 per cent. or more plants infected in 1932, and all these showed a decrease in percentage infection in 1933 varying from 3.4 to 28.7 and averaging from 11.7 per cent. This decreased infection in heavily infected plots suggests auto-intoxication or dying out of the parasite when it becomes very abundant, but it is also possible that in badly affected plots plants were killed off at an earlier stage and had disappeared when the samples were harvested. This question needs further investigation.

Table II.

Soil pH and percentage plants infected by take-all.

Soil pH	Wheat					Barley			
	Plot No.	Percentage plants with take-all				Plot No.	Percentage plants with take-all		
		1931	1932	1933	Average		1932	1933	Average
4.4	—	—	—	—	—	2A	0	1.8	0.9
4.7	—	—	—	—	—	5A	0	0	0
4.7	2A	0	0	0	—	8A	0	0	—
4.8	8A	0	0	0	0	—	—	—	—
4.8	8B	0	0	0	—	—	—	—	—
4.9	2AA	0	0.3	0	—	—	—	—	—
5.0	2B	0	0.4	0	0.1	8B	0	1.0	1.1
5.0	8AA	0.3	0	0	—	5AA	0	3.4	—
5.1	5A	0	0	2.2	2.2	2AA	2.7	5.7	2.4
5.1	8BB	0	2.3	8.4	—	10B	0	1.2	—
5.3	2BB	14.5	35.0	23.0	12.5	—	—	—	—
5.4	10B	0	0	2.4	—	1	8.0	8.1	8.5
5.4	—	—	—	—	—	4A	6.8	10.9	—
5.5	7	13.5	20.6	43.2	—	—	—	—	—
5.6	1	23.4	31.8	35.2	—	7	13.8	8.4	—
5.6	5B	43.8	44.7	16.0	26.8	3B	4.9	12.2	—
5.6	3B	20.0	40.7	29.5	—	10A	6.0	2.3	7.8
5.6	10A	1.9	3.8	34.5	—	11A	13.5	1.3	—
5.7	—	—	—	—	—	4B	13.1	9.2	—
5.7	9B	23.6	24.5	28.5	—	9A	5.5	6.3	—
5.8	4	28.7	36.0	32.6	—	2B	5.1	3.7	—
5.8	3A	30.8	35.0	28.9	—	8AA	9.0	9.6	8.1
5.8	6	17.9	29.8	39.8	30.8	6	9.2	13.0	—
5.8	9A	23.1	52.5	42.7	—	9B	1.8	1.0	—
5.8	11A	9.6	31.8	38.9	—	11B	16.2	10.1	—
5.9	11B	36.1	43.1	33.2	37.5	2BB	3.9	2.7	—
5.9	—	—	—	—	—	5B	8.9	15.1	6.7
5.9	—	—	—	—	—	8BB	3.6	11.5	—
5.9	—	—	—	—	—	3A	1.8	5.8	—
6.3	—	—	—	—	—	3BB	1.3	5.0	4.0
6.4	—	—	—	—	—	3AA	1.5	8.2	—

In barley the percentage of take-all was recorded for only two years, as shown in Tables I and II. Infection increased from 1932 to 1933 in fifteen out of twenty-six plots, and in two plots there was no infection

in either year. Only five plots had more than 10 per cent. plants infected in 1932, and all these showed a decrease in infection in 1933 varying from 2.9 to 12.2 and averaging 6.1 per cent. Four plots with less than 10 per cent. infection also showed a decrease in infection, but this was small, varying from 0.8 to 3.7 and averaging only 1.8 per cent. There

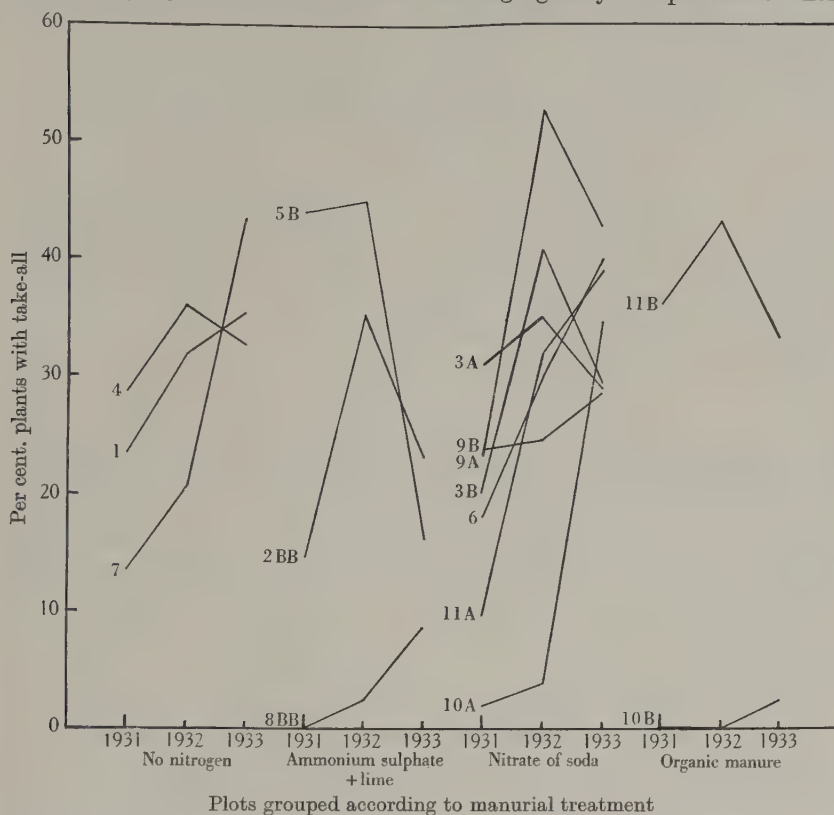


Fig. 1. Continuous wheat. Variation in take-all from 1931 to 1933.

thus appears some tendency, with a few exceptions, for infection in barley to rise to 10 per cent. and, when the degree of infection exceeds this figure, for it to decrease. The percentage infection at which a decrease begins is thus much lower for barley than for wheat, about 10 and 35 per cent. appearing to be the critical degrees of infection for the two crops.

Table III summarises the total numbers of clean and diseased plants taken from the twenty-two wheat plots in the three successive years. The rates of seeding were 3 bushels per acre in 1931 and 1932 and $3\frac{1}{4}$ bushels per acre in 1933. In 1932 there was a slight increase in the

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total number of plants compared with 1931 and a considerable increase in the percentage showing take-all which rose from 13·3 to 22·9 per cent. In 1933 the total number of plants was less than half that obtained from the same number of samples in 1932, but the percentage of take-all remained about the same. The yield of grain decreased each year as manurial residues became exhausted.

Table III.

*Total numbers of plants and yields from all samples
from all wheat plots, 1931-3.*

Year	Number of plants			Percentage plants infected by take-all	Yield of grain in gm.	
	Clean	Infected by take-all	Total		From clean plants	From plants infected by take-all
1931	6794	1045	7839	13·3	2215	82
1932	6797	2022	8819	22·9	1615	220
1933	3283	1033	4316	23·9	1286	148

Loss due to take-all.

An estimate of the loss due to take-all is obtained by deducting the actual yield of diseased plants from the yield of an equal number of clean plants from the same plot and regarding this as the loss due to the presence of disease. This loss was calculated as a percentage of the potential yield which would be expected from clean and diseased plants if all had been free from disease. The average loss in thirteen infected plots rose slightly each year from 16 per cent. in 1931 to 20 per cent. in 1933, while the maximum loss due to take-all in any plot was 42 per cent.

This method of calculating loss assumes no effect of diseased on neighbouring clean plants either by causing a slight infection and so reducing the yield of clean plants, or by reducing competition and so increasing their yield. A statistical examination¹ showed that the presence of diseased plants reduced the yield of neighbouring clean plants in 1931, the effect being just significant at the 5 per cent. level, but showed no effect in 1932 or 1933. No account has been taken, in the estimate of loss, of plants which die and disappear in the early stages of crop life, and for this reason the above estimates may err in being too low.

¹ I am indebted for this to Prof. R. A. Fisher and to the present Statistical Department at Rothamsted.

Other fungi.

Species of *Fusarium* causing stunting and a pinkish coloration of the basal parts of the plant were uncommon on these plots though they appeared to be more plentiful on neighbouring wheat plots where the same crop had not been grown continuously.

A white mycelium belonging to an Agaric of the *Cortinarius* type was found frequently on the roots and basal parts of both wheat and barley. A comparison of yields of clean plants and those affected by the fungus made from figures obtained in 1933 show generally little or no loss associated with the presence of the fungus, presumably indicating that it is not usually actively parasitic. But, in two plots, 5B and 6, where the fungus was plentiful, the yield of affected plants was considerably less than that of clean plants, involving a loss in potential yield of 37 and 18 per cent. respectively and suggesting that, under certain conditions, the "white mycelium" may become parasitic. In plot 5B the general appearance of the plot was worse and the yield less in 1933 than in the previous year, though there had been a considerable decrease in percentage of take-all. There had, however, been a considerable rise in percentage of plants affected by white mycelium, from 13.9 to 61.2 per cent., accompanied by a loss in yield of 37 per cent. associated with the presence of this fungus. This affords some explanation of the deterioration of plot 5B from 1932 to 1933 and suggests that, in some way, the conditions prevailing in this plot may have encouraged the development of parasitism in this fungus.

WHEAT GROWN IN ALTERNATE YEARS IN GREEN-MANURING
EXPERIMENTS.

Wheat is grown in alternate years with a green crop intervening on Stackyard and on Lansome fields. In Stackyard the experiment consists of four plots each divided into a limed and unlimed half. Mustard and tares are grown and fed off by sheep in two plots each year while wheat is grown on the other two, the crops alternating. There are five plots in Lansome, two belonging to the old series begun in 1892 and three to the new series begun in 1921. Wheat is grown on all plots one year and in alternate years the control plot lies fallow, while green manure, mustard and tares, is grown on the other four. A dressing of lime has been applied to one-half of each plot.

The percentage of take-all in 1933 on these plots, shown in Tables IV and V, is considerably less than on most of the continuous wheat

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plots affected by the disease. How far the lower incidence of take-all on these plots depends on the fact that wheat was grown only in alternate years and not every year, on the effect of green manure in place of artificials or farmyard manure, or on other factors is not clear. Further, the *pH* values are higher in most of the green-manured plots than in the continuous wheat. In two green-manured plots, however, *pH* values of 5·3 and 5·7 are found and, in these, the percentage of take-all is insignificant while, in the continuous wheat plots of the same *pH*, considerable infection is found.

Table IV.

*Take-all on wheat grown in alternate years. Stackyard field.
Green-manuring experiment.*

Treatment		<i>pH</i> of soil	Percentage take-all in 1933
Tares:	Unlimed	6·8	13·1
	Limed	7·3	11·8
Mustard:	Unlimed	7·4	9·3
	Limed	7·6	6·5

Table V.

*Take-all on wheat grown in alternate years. Lansome field.
Green-manuring experiment.*

Treatment			<i>pH</i> of soil	Percentage take-all in 1933
New Series. Control:	Unlimed		6·8	10·9
		Limed	7·2	1·7
	Tares:	Unlimed	6·0	13·7
		Limed	6·5	12·4
,, Mustard:	Unlimed		6·3	18·6
		Limed	6·8	6·6
	Tares:	Unlimed	5·7	0·4
		Limed	6·5	2·6
,, Mustard:	Unlimed		5·4	0
	Limed		6·3	8·5

SUMMARY.

Surveys were made in 1931, 1932 and 1933 of the incidence of take-all on wheat and barley grown in the continuous experiments at the Woburn Experimental Station. Ten random samples consisting of all the plants in two half-metre lengths separated by a metre and taken from adjacent rows were obtained from each plot. The numbers of clean and diseased plants, their tillers and yield of straw and of grain were determined for each sample. No manure had been applied since 1925–6 except to certain of the barley plots.

There was little or no take-all on plots which had received ammonium sulphate alone, with minerals, or with 1–2 tons of lime, while the disease was present in most of the other plots.

In general there was a higher percentage infection on wheat, reaching a maximum of 52.5 per cent. on one plot, than on barley in which the highest percentage infection on any plot was 16.2.

Little or no take-all appeared on either wheat or barley in plots whose pH was 5 or less, comparatively little at pH 5.1 and in general considerably more at higher pH values. In spite of considerable variation in infection at the same pH values there was a general tendency for percentage infection to rise with pH in wheat, but this was not apparent in barley.

The wheat plot 10B which had received rape dust was exceptional in that though it had a pH of 5.4 little infection appeared. The corresponding barley plot also had little infection but its pH was only 5.1.

No clear relationship between yield and incidence of take-all was found.

In the three years in which observations were made take-all appeared to increase in wheat till 35 per cent. of the plants were infected and then to decrease. Two years' observations on barley suggest that the corresponding figure for this crop is about 10 per cent.

The total number of plants obtained from all the wheat samples increased slightly from 1931 to 1932 and decreased by more than half in 1933. The average percentage of take-all increased considerably from 1931 to 1932 and remained about the same in 1933.

The average loss in yield due to take-all in thirteen affected plots rose from 16 per cent. in 1931 to 20 per cent. in 1933, with a maximum loss in any plot of 42 per cent.

A white mycelium belonging to an Agaric of the *Cortinari* type was found frequently on the roots and basal parts of wheat and barley. In general it did not appear parasitic but there is some evidence that in certain plots it had become so, causing considerable loss in yield.

Take-all was very much less on wheat grown in alternate years with green manure intervening than on the continuous wheat.

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A VIRUS DISEASE OF *PRIMULA OBCONICA* AND RELATED PLANTS

BY KENNETH M. SMITH, D.Sc., Ph.D.

(Potato Virus Research Station, School of Agriculture, Cambridge.)

(With Plate XVII.)

A PLANT of *Primula obconica* in a badly diseased condition was recently received from Mr Donald E. Green of the Laboratory of the Royal Horticultural Society at Wisley, who informed the writer that no organism responsible for the disease in the *Primula* could be found. Some inoculations were therefore made from the plant to various test plants on the assumption that a virus might be concerned in the disease. This proved to be the case and the results of the inoculations are shortly given herewith.

SYMPTOMS OF THE VIRUS IN DIFFERENT HOST PLANTS.

Primula obconica.

The symptoms on the plant of *P. obconica* as originally received were mainly necrotic though there was some dark green mottling on the younger leaves. The necrosis continued to develop until the plant was practically killed (Pl. XVII, fig. 1). It was found possible to transmit the virus to young plants of *P. sinensis* and these developed a disease closely similar to that shown by *P. obconica*. The rubbed leaves became discoloured and often necrotic while the younger leaves developed a faint dark and light green mottle, the darker colour being associated with the veins, giving a vein-banding effect. Infected plants made very little growth.

Nicotiana tabacum (var. White Burley).

Inoculation from the diseased *Primula* to young tobacco seedlings produced a few large lesions on the rubbed leaves in about 5-7 days. The lesions were not circular but irregular in shape and involved both upper and lower surfaces of the leaf giving a glassy appearance to the affected parts. As a rule these lesions did not develop on inoculation to older plants, but the inoculated leaves became generally chlorotic and unhealthy in appearance. Systemic infection developed some days later, the young leaves showing a fairly distinct mosaic mottle of dark and light green with occasional small necrotic spots (Pl. XVII, fig. 3).

Nicotiana glutinosa.

Symptoms appeared on *N. glutinosa* 7-10 days after inoculation. The first sign was a faint yellowish mottle on the youngest leaves, later the whole plant took on a paler colour than normal. The youngest leaves remained yellowish and upright with a slight tendency to curl inwards. These symptoms were followed later by the production of a number of adventitious shoots and the whole plant exhibited a rosetted and dwarfed appearance (Pl. XVII, fig. 2).

Nicotiana langsdorffii.

On *N. langsdorffii* a faint mosaic mottle on the youngest leaves was the only symptom produced.

Datura stramonium.

On *Datura* the incubation period seemed rather longer than in the foregoing plants. The first sign of the disease was a systemic infection appearing either as a mottle or as a number of chlorotic rings with a darker green centre (Pl. XVII, fig. 4). The mottling usually consisted of a speckling of darker green on a lighter background.

COMPARISON OF THE *PRIMULA* VIRUS WITH A STRAIN OF
CUCUMBER MOSAIC VIRUS.

The type of symptom induced in *Nicotiana glutinosa* by inoculation from the diseased plant of *Primula obconica* was very reminiscent of the symptoms produced by cucumber virus I in that plant. The most striking similarity was in the production of secondary shoots (Pl. XVII, fig. 2). Some tests were therefore carried out to see if the two viruses were identical. There was available for comparison a strain of cucumber virus I, kindly sent to the writer by Mr L. Ogilvie who obtained it from vegetable marrows. The symptoms produced by these two viruses on *N. glutinosa* were so similar as to be almost indistinguishable from each other, and the same may be said of the symptoms on White Burley tobacco. In addition both viruses produced similar local lesions on leaves of cow-pea (*Vigna sinensis*).

The resistance to ageing in extracted sap was also compared with that of cucumber mosaic virus and tested on cow-pea. In both cases the longevity *in vitro* at room temperatures was less than 96 hours.

From the above tests it seems fair to conclude that the virus affecting the *Primula* was actually a strain of cucumber mosaic. Furthermore, inquiry elicited the fact that the diseased primulas had been grown in the same frame with a number of young vegetable marrow plants, and these plants had shown symptoms of mosaic disease when planted out later in the season.

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It is possible that the virus of cucumber mosaic is responsible for diseases of other plants of horticultural importance. Last year some spinach plants were received from Dr Dillon Weston and these showed symptoms much resembling the disease of "spinach blight" which has been proved by Hoggan (1933) to be due to a virus of cucumber mosaic. Inoculation from these spinach plants to White Burley tobacco produced a disease somewhat similar to that caused in the same plant by inoculation with cucumber virus I.

Not long ago the writer observed some *Datura* plants, growing in the middle of a potato field on the University Farm at Cambridge, which were infected with the virus of cucumber mosaic, the symptoms in the *Datura* being closely similar to those illustrated in Pl. XVII, fig. 4. In this case the virus appeared to have been brought to the *Datura* by winged individuals of the aphid *Macrosiphum gei*.

SUMMARY.

A virus disease of *Primula obconica* is described. The virus is shown to be transmissible to various Solanaceous plants on which it produces symptoms closely resembling those caused by cucumber virus I on the same host plants. Comparison of the virus from the *Primula* with a strain of cucumber virus I suggests that the two are very similar if not identical.

ACKNOWLEDGMENT.

The writer has pleasure in acknowledging the assistance given by Mrs D'Oliveira in the course of this work.

REFERENCE.

HOGGAN, I. A. (1933). Some viruses affecting spinach and certain aspects of insect transmission. *Phytopath.* XXIII, 446-74.

EXPLANATION OF PLATE XVII.

Fig. 1. Plant of *Primula obconica* in an advanced state of the disease.

Fig. 2. Plant of *Nicotiana glutinosa* inoculated from the *Primula* shown in Fig. 1. The leaves are paler than normal and curved upwards. Note the numerous adventitious shoots.

Fig. 3. Symptoms produced in White Burley tobacco by the *Primula* virus. Systemic infection may be seen in the upper and lower leaves on the left of the figure.

Fig. 4. Symptoms produced in *Datura stramonium* by the *Primula* virus, in this case chlorotic rings and lines.

(Photographs by J. P. Doncaster.)

(Received January 14th, 1935.)



SMITH.—A VIRUS DISEASE OF *PRIMULA OBCONICA* AND RELATED PLANTS (pp. 236-238).

A VIRUS DISEASE OF CULTIVATED CRUCIFERS

BY KENNETH M. SMITH, D.Sc., Ph.D.

(Potato Virus Research Station, School of Agriculture, Cambridge.)

(With Plates XVIII and XIX.)

IN the autumn of 1934 in the neighbourhood of Cambridge a number of cabbages, brussels sprouts and other brassicas were observed to be affected with a disease showing unfamiliar symptoms. Inoculations from these affected plants proved that the disease was infectious. Moreover, it appeared to be of a virus nature. Since the disorder does not seem to have been described before, this paper is published, giving a short account of its symptomatology on various plants. Studies on the insect vectors of the virus are still in progress.¹

SYMPTOMS OF THE DISEASE ON DIFFERENT HOST PLANTS.

Brassicas.

The symptoms as observed in the field upon full-grown cabbages and brussels sprouts plants are mainly of a "ringspot" nature. The leaves, particularly the older leaves, are covered with necrotic rings uniformly distributed over the surface. The rings are almost black in colour and deeply sunk in the tissue (Pl. XVIII, fig. 1). In smaller plants the necrosis may take the form of circular or irregular lesions. The symptoms are somewhat reminiscent of certain bacterial diseases(2) except that there is no blackening of the veins characteristic of attack by *Pseudomonas campestris* for example, and the rings are too numerous and uniformly distributed. Furthermore, bacteriological examination has failed to reveal the presence of any causative organism.

The disease is transmissible to cabbage by sap transfusion. A high percentage of positive infections has also been obtained with inoculations from infected brassicas to various plants of the Solanaceae. In Pl. XVIII, figs. 2, 4 and 5, are shown two young cabbage seedlings which have been experimentally infected with the disease. Experiments are now in progress to examine the effect of this virus on certain other cruciferous crops, particularly turnips and swedes.

¹ The insect vector has since been proved to be the aphid *Myzus persicae* (Sulz.).

Nicotiana glutinosa.

While investigating the host range of this virus some inoculations were made to young plants of *N. glutinosa*, the source of inoculum being a naturally infected brussels sprouts plant from the field. Inoculations were made by means of glass spatulas. Local symptoms developed in 10–16 days, large necrotic lesions appearing on the inoculated leaves (Pl. XIX, fig. 6). These lesions were either in the form of necrotic patches or of rings with a necrotic edge. The development of symptoms was fairly rapid after the appearance of the first lesions.

In young seedlings the disease spread through the whole leaf, passed into the stem and appeared systemically in the young leaves in the form of gross necrotic lesions. Occasionally a slight mottle developed, but usually death ensued before this. In older plants the inoculated leaves were destroyed similarly by necrosis, but a mottle of light green and yellow flecks appeared on the youngest leaves. As a rule the virus was fatal to *N. glutinosa* (Pl. XIX, fig. 7), but plants that survived developed a striking yellow and green mosaic mottling.

Three separate lots of inoculations were made from different affected brassicas in the field to *N. glutinosa* and in every case some infections resulted.

CROSS-INOCULATION STUDIES.

It was found easy enough to transmit the virus from infected to healthy *N. glutinosa* plants, and as a rule 100 per cent. infection was obtained. Inoculations were also made to *N. glutinosa* plants from the seedling cabbage plants experimentally infected by inoculation from the diseased field brassicas and again the same disease resulted.

Three series of healthy cabbage seedlings were then inoculated from the diseased *N. glutinosa* plants. Symptoms developed in most of the seedlings 20 days later, the incubation period being somewhat longer in the cabbage than in the Solanaceous plants. As a rule necrosis developed on the inoculated leaves of the cabbage seedlings. Systemic infection showed in the younger leaves as a mosaic mottle which gave a marbling effect. There was no preliminary clearing of the veins such as appears to be characteristic of infection with cabbage mosaic. Necrotic rings developed later in the young cabbages experimentally infected, and occasionally chlorotic rings also developed. Inoculation from these cabbages back to *N. glutinosa* reproduced the necrotic disease in these plants.

Nicotiana tabacum (var. White Burley).

Necrotic lesions develop on the inoculated leaves of White Burley tobacco in 7–10 days (Pl. XIX, fig. 8). The lesions increase in size and occasionally spread through the leaf. So far, however, the virus has not become systemic in tobacco plants of this variety.

Nicotiana langsdorffii.

Circular local lesions appear on the inoculated leaves of *N. langsdorffii* in 10–12 days; the necrosis destroys both upper and lower epidermis, giving a clear and glassy appearance to the lesions. Systemic infection follows in the form of a well-marked light green or yellow mottle on the young leaves together with some distortion. The disease was less severe on *N. langsdorffii* than on *N. glutinosa* (Pl. XVIII, fig. 3).

COMPARISON OF THE DISEASE WITH MOSAIC OF CRUCIFERS.

A mosaic disease of cruciferous plants is widespread in the Cambridge district. It can be found affecting various cultivated garden brassicas and it is also common in fields of kale. Since Clayton⁽¹⁾ states that the symptoms of mosaic of crucifers can take on a ring- or horseshoe-like appearance in brussels sprouts plants, a few experiments were performed with this virus to see if the ringspot and mosaic diseases were due to the same cause. The mosaic virus was found to be easily transmissible by inoculation among different varieties of brassicas. In every case the first sign of infection was a pronounced clearing of the veins. The symptoms of the disease usually consisted of a mottling, though there was a good deal of distortion and rosetting of the plant in some cases. Inoculation to *N. glutinosa* has so far failed to produce any infection.

The facts that the ringspot virus apparently never causes clearing of the veins in brassicas and that the mosaic virus is not transmissible to *N. glutinosa* are considered sufficient to show that the mosaic of cruciferous plants and the disease described in the present paper are probably due to different viruses. It is, however, possible that more than one virus is concerned with the ringspot disease.

COMPARISON OF THE VIRUS WITH THAT OF TOMATO SPOTTED WILT.

In view both of the wide host range of the spotted wilt virus and its ever-increasing spread through horticultural plants of all kinds, the possibility had to be considered whether the disease under study might not be due to this virus. A careful comparison, however, of the symptoms

caused by the two viruses respectively upon *N. glutinosa* shows that the two are distinct. Furthermore, tests of their physical properties, so far as they have been carried, also support this view.

SUMMARY.

An account is given of an apparently undescribed virus disease affecting cultivated crucifers and other plants. The virus is sap-transmissible and has been so transmitted to young cabbages and also to various members of the Solanaceae. On *Nicotiana glutinosa* it produces a disease which is usually fatal.

Preliminary tests with the virus differentiate it from the viruses of tomato spotted wilt and cabbage mosaic.

ACKNOWLEDGMENTS.

The writer has pleasure in acknowledging the assistance given in this work by Mrs D'Oliveira. The plants of *N. langsdorffii* used in the experiments were grown from seed kindly supplied by Dr L. O. Kunkel.

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- (1) CLAYTON, E. E. (1930). A study of the mosaic disease of crucifers. *J. agric. Res.* XL, 263-70.
- (2) McCULLOCH, L. (1911). A spot disease of cauliflower. *Bull. U.S. Bur. Pl. Ind.* No. 225, 1-15.

EXPLANATION OF PLATES XVIII AND XIX.

PLATE XVIII.

- Fig. 1. The "ringspot" type of symptom on cabbage leaf from an old infected plant in the field.
- Fig. 2. Young cabbage seedling infected by inoculation from a diseased plant of *N. glutinosa*. Note the necrosis on inoculated leaf on the left.
- Fig. 3. *N. langsdorffii* showing systemic infection. Circular lesion on inoculated leaf. Note mottle on central leaves.
- Figs. 4 and 5. Leaves from a cabbage seedling experimentally infected, enlarged to show necrosis and type of mottling.

PLATE XIX.

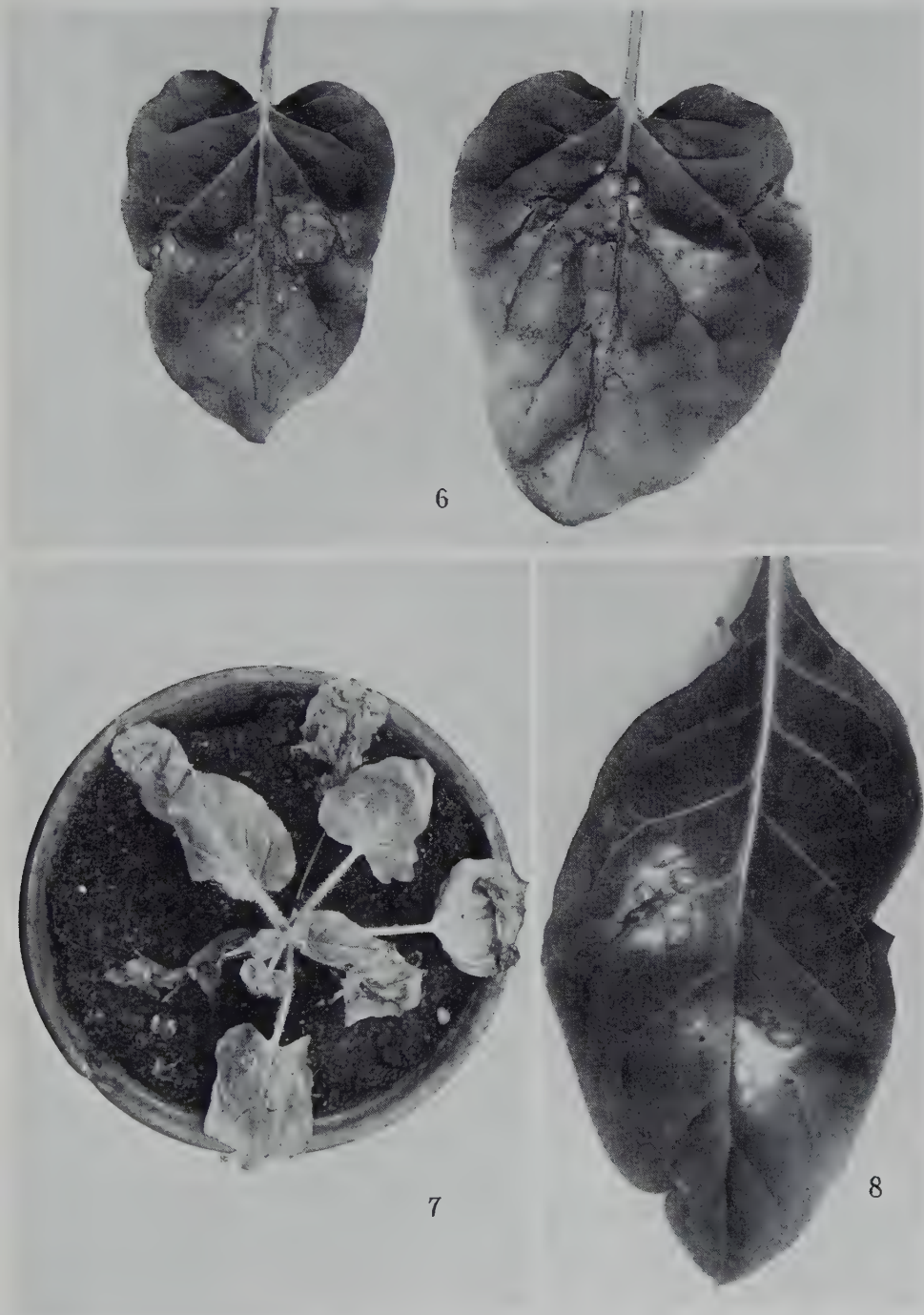
- Fig. 6. Inoculated leaves of *N. glutinosa* showing the type of local lesion which develops.
- Fig. 7. Plant of *N. glutinosa* killed by the virus from a naturally infected cabbage plant in the field.
- Fig. 8. Local lesions on an inoculated leaf of White Burley tobacco.

(Photographs by J. P. Doncaster and C. W. Williamson.)

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SMITH.—A VIRUS DISEASE OF CULTIVATED CRUCIFERS (pp. 239-242).

FURTHER EXPERIMENTS ON THE ARTIFICIAL FEEDING OF *MYZUS PERSICAE* (SULZ.)

BY M. A. HAMILTON, PH.D.

(*Department of Plant Pathology, Rothamsted Experimental
Station, Harpenden, Herts.*)

(With 1 Text-figure.)

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INTRODUCTION.

THERE are several problems concerned with transmission of virus diseases by insects in which progress is seriously impeded by lack of information about the vectors themselves, such as the simple mechanics of feeding and the circulation of foodstuffs and foreign bodies in the blood stream. For instance, there is no reason, except supposition, to believe that it is even possible for foreign bodies or solutions to be absorbed in the stomach, circulate in the blood and reach the salivary glands in aphids, though this has been demonstrated for leaf-hoppers(7). In small and delicate insects such as the aphids this information is very difficult to obtain. They have little resistance to surgical operations such as injection and vivisection and, a very serious check to the success of direct methods,

the extracted juices of the aphids, with or without the juices of the salivary glands, have a deleterious effect on the viability of the virus. For example, a given quantity of L_1 filtered virus juice, 1 gm. leaf to 10 c.c. water, is apparently inactivated by an equal quantity of centrifuged aphid extract obtained from 1 gm. of aphid bodies to 3 c.c. of water.

These considerations make it necessary that work on these problems should be carried out with living insects. However, attempts to introduce the virus by artificial feeding, following the examples of Storey (6) and Carter (1) who were successful with leaf-hoppers feeding on extracted juices of curly top of sugar beet and streak disease of maize, respectively, were with Hy. III and also with potato virus Y of K. M. Smith (5) unsuccessful. Aphids have been fed on undiluted and frequently changed infectious plant extracts and placed on healthy seedlings at the rate of 100 per plant, without any plants becoming infected. As will be seen from results obtained by the use of dyes and other indicators there is every chance of a sufficient amount of liquid being imbibed by such a number of aphids, even under the favourable conditions of artificial feeding, and the reason for this lack of success is obscure. The only hypothesis which can at present be suggested is as follows:

The mouth-parts of aphids contain two separate channels, an inhalant one passing into the alimentary canal and an exhalant one passing from the salivary glands, and if the virus does pass through the body of the insect it is bound to pass through both those channels to complete the circuit from the source to the object of infection.

The inhalant channel is relatively large, its lumen can be seen plainly in section under an oil immersion lens and appears in fixed specimens to be about 1μ in diameter. The exhalant channel is much smaller, its lumen cannot be resolved under the oil immersion lens and it appears in section as a minute dark speck. It cannot therefore be greater than about $0.2-0.3\mu$ in diameter. According to Henderson Smith and McClement (4) the size of the Hy. III virus particle in an aqueous solution is about 0.15μ , but, in filtration through a Chamberland filter without any preliminary treatment (except graded filtration), this virus cannot pass through the L_3 filter, in which the passages are larger than this, except when very large quantities are passed. Even then a considerable amount is held back, which can be recovered in a viable state from the inside of the candle by washing. This suggests that the particles suspended in the fresh plant extract become coated with precipitated plant protein or, in some other way, become too large to pass out of the insect down the narrow ejaculatory duct. Unfortunately it is impossible to test the insect

juices for the presence of virus, which presumably could be imbibed up the large inhalant duct, because of the deleterious effect of the insect juices, after extraction from the insect, on the virus.

If the virus is carried on the outsides of the stylets and inoculated mechanically, there seems to be no reason why infection should not be obtained by artificial feeding, and this is a very strong argument against that possibility.

Thus two lines of approach to the solution of what might be termed the mechanical problems of insect transmission are, at present, closed. There remains a third method of attack, the least direct and the most susceptible to errors of experiment and the pitfalls of false analogy, but one which, given sufficient knowledge of insect and mechanical infections under normal conditions, may be made to yield a great deal of information on obscure points.

It consists in using, instead of virus juices, an indicator, which may arbitrarily be taken to represent the virus, and tracing its course from source to object as though it were a known dilution of virus. Naturally, with such a method, negative results have no significance because we cannot assume that the indicator will act in the same way as the virus, but, if the indicator does give the same results as we should expect the virus to do under equal conditions, then it is confirmatory evidence that the virus goes through the expected series of movements. Also, physiological data can be obtained which, while having no direct connection with virus behaviour, are of importance in increasing our very small knowledge of the quantities and conditions of fluids which may reasonably be supposed to represent those normally acting as vehicles for the virus.

AIM OF INVESTIGATION.

The investigation had three main objectives to determine:

- (1) The volume of liquid imbibed by aphids when feeding.
- (2) Whether any foreign body in true or colloidal solution would be passed through the blood stream into the salivary glands and ejected into the feeding substance.
- (3) The quantities of a foreign body thus ejected and the volume of liquids involved in the ejection of these quantities.

METHODS.

Technically the investigation involved two main problems:

- (1) To find a technique by which the aphids might be induced to feed with a reasonable degree of consistency.

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(2) To find a substance, not injurious to them, which it is possible to detect in solution in very minute quantities of liquid.

(1) *The feeding capsule.*

In an earlier paper⁽³⁾ the writer described preliminary attempts to solve the first problem, but the method described did not give sufficiently consistent results to be practicable for this investigation. Therefore further experiments were made using variations of these methods. Instead of large capsules small glass rings were used in the manner described by Roach and Massee⁽⁸⁾, and, instead of animal membranes, the actual feeding surface was formed by the epidermis of cabbage leaves from which the leaf tissue had been removed.

The rings are sections of glass tubing about 2 cm. in diameter and 1 cm. deep. The epidermis is prepared by clamping a piece of cabbage leaf, upper surface downwards, on to a wooden disc covered with thick blotting paper, and gently scraping with an ivory spatula. Iris or tulip leaf is equally good for the purpose, but does not give such a large area. The upper surface is used despite the fact that aphids usually feed on the lower one, because it is stronger and more waterproof. The cabbage plants are young succulent plants with leaves about 4–6 in. long. The rings are prepared by dipping one edge into molten paraffin wax and the epidermis is sealed on, dry surface inwards, with a warm spatula. They are then enclosed in inverted sporulating dishes, the lid of the sporulating dish acting as a base for the capsule.

Various types of medium, nutrient agars and broths, were tested as vehicles for the indicators, but as the object was not to rear aphids but to make them feed for short periods comparable with those involved in virus transmission, it was found advisable to keep the medium as simple as possible. The one eventually decided upon, as giving the best and most consistent results, was 3 per cent. agar with 20 per cent. saccharose. The agar was required to prevent the passage of the solution through the membrane when it was pierced by the insects, and was used in sufficient concentration to allow the medium to be diluted by half, when the indicator solutions were added to it.

(2) *The indicator.*

Some account has already been given of the use of dyes and vital stains as indicators for the length of feeding periods, but it is difficult to detect these at all in very small quantities, and impossible by any practicable means to estimate the actual amount present in the insects.

Some attempts were made with red gold sols, as gold can be detected in very high dilution, but the solutions were always precipitated, possibly by the action of the salivary juices of the insects.

The indicator finally decided upon, which has proved most expedient, was a radioactive substance. In this part of the work very valuable advice and practical assistance was received from Dr J. Chadwick of the Cavendish Laboratory, Cambridge, to whom I am greatly indebted. At his suggestion the feeding solution was made up to contain a weak solution of radium (D + E + F). The actual indicator was the radium F or polonium contained in the solution. Since polonium emits α -particles its presence can be readily detected by means of a simple gold-leaf electroscope. The rate of discharge of the electroscope gives a measure of the amount of polonium present.

Using polonium solution as an indicator the technique is as follows:

The medium is made up as described with the addition of an approximately equal volume of polonium solution. It must be kept slightly acid to keep the polonium in solution. A drop of the medium is placed on the outer surface of each capsule and allowed to set. The aphids are then inserted beneath with a camel-hair brush, about 12–20 per capsule, and the whole batch of some 15–20 capsules in their sporulating dishes are placed in a moist chamber and the aphids allowed to feed for a definite period of time. In the earlier experiments the medium was also coloured with various intravital stains, so that by observing the presence of dye in the aphids it could be ascertained what proportion had fed. This method was abandoned, as it entails individual examination of the aphids, and the dyes used were liable to precipitate in the agar bringing the polonium down with them, unless the pH was very carefully adjusted. The number of aphids which could be observed from this test to have fed, varied considerably. The actual mean was 23 per cent., but many probably imbibed amounts too small to be detected. When dealing with the volume of liquid ejected by the aphids into the leaf on the second feeding, a control consisting of a few aphids from each capsule was always set up.

When the aphids had fed for the required time they were either killed by chloroform and macerated in nitric acid immediately, or else a number of them were placed on small *Hyoscyamus* leaves in Petri dishes closed by cellophane covers for a range of periods varying from 12 to 48 hours; these being the periods over which a parallel series of actual virus infections on plants was being conducted.

The aphids were then removed from the leaves, both were tested

under the electroscope, and aphids and leaves separately underwent the same treatment of maceration as the controls.

After one or two days in nitric acid the solution of leaves or aphids was spread out on the bottom of a Petri dish and dried on a hot plate. The plate was then ready for testing. At the same time a test plate was also made containing 0.01–0.05 c.c. of the same polonium-agar medium on which the original insects were fed; by comparison with this plate the quantities are calculated.

Before maceration, the outsides of the aphids and the leaf surfaces were carefully tested for radioactive solution which might have leaked through the feeding membrane on to the feet of the aphids, or been excreted by them on to the leaf. Any sign of such discharge was recorded and corrected for in the final result.

The actual measurement of the α -particle activity of the dried film, containing polonium, is made in a simple type of gold-leaf electroscope.

This consisted of a small metal vessel with a movable bottom in which the Petri dish was placed. The gold-leaf system was of the usual type and was charged to a potential of about 300 volts. The deflection of the leaf is approximately proportional to the voltage. The gold-leaf system will lose its charge, and the deflection of the leaf will decrease, owing partly to leak across the insulation, partly to the ionisation of the air in the electroscope. The rate of fall of the leaf is observed by means of a low-power microscope provided with a scale in the eyepiece. When no radioactive matter is present the rate of fall of the gold leaf is very small. This rate is called the "natural leak". When active material, *e.g.* the dried film from aphids which have fed on the radium (D + E + F) solution, is placed in the bottom of the electroscope, the α -particles emitted by the polonium produce an ionisation current which causes a more rapid rate of fall of the gold leaf. The rate of fall, corrected for the natural leak, is proportional to the ionisation current and therefore also to the amount of polonium present in the film.

(3) *Weighing experiments.*

Another series, closely connected with the polonium feeding, were the weighing experiments. In these a number of aphids (generally 100) were weighed in a weighing bottle, transferred to covered Petri dishes, starved for a definite number of hours and reweighed to obtain the loss from starvation, then fed on *Hyoscyamus* or other leaves for a short time (generally 6 hours) until they had just not made up their original weight. Probably the actual amount taken up was greater than the figures ob-

tained, because some would certainly be lost by excretion and respiration, but it was assumed that the starving aphids would use most of the material which they imbibed for restoring their tissues before losing very much. Thus the figures obtained would give an idea of the amounts imbibed during that period by a different method from that of polonium feeding, and would serve as a check on the more complicated experiments.

A parallel series of aphids were artificially fed on various media and weighed in the same way, but, as will be seen from the results, this method was not very successful for several reasons.

RESULTS.

(1) *Volume of liquid imbibed by the aphid.*

The method of calculating the results, from the data obtained from the feeding experiments on polonium-agar solutions, is shown in the following example from one experiment. The results were always expressed in terms of 100 aphids although the number actually feeding could not be determined; only living aphids were tested except in special cases. The words in brackets are terms used for the sake of brevity in referring to the various items.

Experiment.

1st day.

100 aphids fed on polonium-agar medium for 24 hours.

2nd day.

80 aphids surviving.

Discharge from outside of bodies of 80 unmacerated aphids after feeding (external discharge) + leak = 0.165 d./m.

Atmospheric leak of electroscope (leak) = 0.14 d./m.

Therefore corrected external discharge = 0.025 d./m.

d./m. = discharge per minute expressed in micrometer scale divisions.

Bodies macerated 24 hours in nitric acid and dried as film.

3rd day.

Discharge from macerated bodies (actual discharge) = 1.81 d./m.

Correction for leak and external discharge = 0.165 d./m.

Corrected actual discharge = 1.645 d./m.

Discharge from dried film of 0.01 c.c. feeding medium (test plate) = 75.0 d./m.

Therefore volume of liquid imbibed by 100 aphids = $\frac{0.01 \times 1.645 \times 100}{80 \times 75} = 0.27 \text{ c.mm.}$

Where quantity of media on test plate = Q .

Discharge from test plate = D .

Corrected actual discharge from aphids = d .

Number of aphids = N .

Volume of liquid imbibed by 100 aphids = $\frac{Q \times d \times 100}{DN}$.

Table I shows the details and results of a series of similar experiments. The high variation between experiments is probably due to differences

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in environmental conditions, and in the condition of the aphid colonies from which the subjects were taken. A spell of hot weather often causes a reduction in the virility of a colony which is reflected in their lack of resistance to artificial conditions, and there are many such influential factors which are difficult to control. The average is 1.05 c.mm. per 100 aphids—the individual results varying from 0.26 to 2.9 c.mm., but a great many attempts gave negligible results. The standard error for a single experiment is 0.91 c.mm.

Table I.

Volume of liquid imbibed by aphids on simple polonium-agar mixtures.

Time hours	No. aphids	d./m.	Quantity on test plate		c.mm. imbibed per 100 aphids
			c.c.	d./m.	
24	40	0.41	0.01	35	2.90
24	100	4.64	0.05	187	1.20
24	60	0.79	0.01	23	0.57
24	80	9.75	0.05	222	2.60
18	44	0.77	0.01	50	0.35
12	40	0.75	0.02	70	0.53
24	60	1.18	0.01	23	0.85
12	60	1.79	0.01	23	1.29
12	300	11.73	0.01	60	0.65
12	40	0.775	0.05	260	0.37
12	130	2.7	0.01	74	0.26

There is a general indication that the quantity imbibed depends upon the time of feeding. The mean quantity for 12 hours' feeding is 0.69 c.mm., and for 24 hours' feeding 1.38 c.mm., but the standard error is 0.357, and these figures are therefore not statistically significant.

Table II gives the effects of various dyes and buffered solutions on the percentage feeding and the volumes of liquid imbibed. In view of the discrepancies in Table I, where the media were as nearly as possible constant, it is not possible to draw any definite conclusion, but there is a general indication that certain dyes such as crystal violet, unless well buffered, give a lower result than more neutral stains.

On the whole these results are lower than those in Table I, for it must be borne in mind that, though some of them seem to be as high, they refer to aphids which are known to have fed, the non-feeders being eliminated. Those of Table I include all the aphids still active in the capsule at the end of the feeding period, and therefore include a good many of the B class shown in Exps. VIII and IX, though perhaps not as many as in these two experiments, because there is no doubt that the dyes have a discouraging effect. By non-feeders is meant those aphids which, whether they actually penetrate the membrane with the stylets or not, do not imbibe sufficient dye solution for this to be detected in

Table II.

Effect of different dyes and varying pH on volume of liquid imbibed.

(Time 24 hours, except No. XIV.)

Exp.	Dye used	No. aphids	d./m.	Quantity on test plate c.c.	d./m.	c.mm. imbibed	pH medium
III	T.R.	50	0.47	0.05	161	0.29	Buffered down to 4.2
IV	N.R.	50	1.9	0.05	170	1.60	6.24 buffered
V	C.V.	20	0.35	0.05	170	0.50	Buffered 6.1
VI	N.R.	80	1.5	0.05	88	1.06	Unbuffered 5.1
VII	M.B.	40	0.5	0.05	200	0.35	Unbuffered 5.3
VIII	M.B. A	20	0.22	0.05	142	0.39	Unbuffered 5.7
	B	50	0.11	0.05	142	0.08	"
	C	60	0.18	0.05	142	0.107	"
IX	C.V. A	20	0.06	0.05	150	0.10	Unbuffered 3.5
	B	50	0.11	0.05	150	0.073	"
	C	50	0.00	0.05	150	0.000	"
XII	M.B. A	6	0.46	0.01	33	2.5	Buffered 6.23
	B	145	0.8	0.01	33	0.18	"
XIII	N.R.	20	0.23	0.001	2	0.57	24 hours
	(gum arabic)						
XIV	N.R.	10	0.20	0.005	4	2.50	48 hours

A=living aphids with stained gut.

T.R. = trypan red.

C.V. = crystal violet.

B=living aphids without stain.

N.R. = neutral red.

M.B. = methylene blue.

C=dead aphids.

Table III.

Quantities actually imbibed by stained aphids.

No. of aphids	Treatment	Quantity imbibed c.mm.
6	M.B. buffered	0.14
10	N.R. 48 hours	0.25
20	C.V. unbuffered	0.02
20	M.B. unbuffered	0.077
20	C.V. buffered	0.097
20	N.R. gum arabic	0.109
40	M.B. unbuffered	0.125
50	T.R. buffered	0.14
50	N.R. buffered	0.81
80	N.R. unbuffered	0.85

the gut. In actual fact most of the aphids, being in a starved condition, do penetrate the membrane, though only a certain percentage of them continue to feed. This can be observed under the dissecting microscope. On almost every occasion if the upper surface of the membrane be observed through the drop of medium, the stylets of the aphids can be seen appearing very soon after the aphid is placed beneath it. Table III gives the quantities actually imbibed by the stained aphids without translation into terms of 100. It shows a general increase in quantity with increasing numbers of aphids but the effect is frequently masked by the varying treatments.

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When the solutions are properly buffered, as in Exps. IV and V, results are better and the figures compare favourably with those of Table I. The basic potassium phosphate and other salts used to buffer the solution often had a deleterious effect on either the agar or the dyes, which have a tendency to precipitate out if the *pH* is changed. For this reason a great many experiments have had to be discarded, as the precipitate brings down polonium, which ceases to be available to the aphid except in very small quantities.

When the *pH* is not adjusted, even with methylene blue the percentage of aphids feeding is low and the results poor. Exps. VIII, IX and XII show very clearly that the figure obtained from feeding a number of aphids is produced by one set which have fed considerably and another, generally a larger batch, which have fed little or not at all. Exp. IX in which crystal violet is used is a good example of the effect of low *pH*.

These results suggest explanations of why the amounts imbibed by the aphids in artificial feeding are lower than one expects from natural feeding, as determined by weighing. It also shows that the results are affected by relatively small differences in the feeding medium; and gives an idea of the delicacy of adjustment of these and other conditions, which would be necessary to reduce the experimental error.

The fact that the use of different dyes is mainly responsible for the change in *pH*, and not other unknown factors such as decompositions of the epidermis feeding membrane, is demonstrated by the following list of the results of *pH* tests on various media:

<i>pH</i> of agar: 20 % sugar solution	=6.93
<i>pH</i> of saccharose agar + polonium solution (1 : 1)	=6.1-6.53
<i>pH</i> of agar polonium + 1 % trypan red (2 : 1)	=7.5
<i>pH</i> of agar polonium + 1 % trypan blue (2 : 1)	=7.2
<i>pH</i> of agar polonium + 1 % methylene blue (2 : 1)	=5.64
<i>pH</i> of agar polonium + 1 % neutral red (2 : 1)	=5.1
<i>pH</i> of agar polonium + 5 % crystal violet (2 : 1)	=3.7
<i>pH</i> of agar polonium + 2 % bismarck brown (2 : 1)	=2.9

The polonium itself does not seem to have any immediate deleterious effect upon the insects: the percentage of feeding is as high with polonium as without. Indeed the first effect seems to be a considerable increase in reproductive activity, though the young ones do not survive very long.

It is interesting to note that though the amount of radium detected on the outsides of the bodies and on the leaf is relatively small, compared with the actual discharge after maceration (Table IV), yet the amount obtained from macerated cast skins (exuvia) as in Exps. 8, 9 and 11

is fairly large. In these about 40-50 young aphids were fed at the same time as the adults, and ecdysed during the experiment.

Table IV.

Comparisons between actual and external discharge per 100 aphids, also of cast skins and from leaf.

Exp. No.	Atmo-spheric leak	External d./m. cast skins per 100	Actual d./m. cast skins per 100	External d./m. aphids per 100	External d./m. leaf	Actual d./m. aphids per 100	Actual d./m. leaf
1	0.09	—	—	0.216	—	1.9	—
2	0.09	—	—	0.19	—	3.8	—
3	0.1	—	—	0.16	—	1.5	—
4	0.1	—	—	0.1	—	0.22	—
5	0.08	—	—	0.11	—	0.66	—
6	0.1	—	—	0.00	—	0.23	—
7	0.125	—	—	0.10	0.00	—	0.125
8	0.08	0.14	0.41	0.12	0.08	0.79	0.125
9	0.07	0.11	0.33	0.07	0.16	9.75	0.5
10	0.01	—	—	0.15	0.07	1.79	0.25
11	0.2	0.09	0.65	0.10	0.03	4.64	0.3

These figures suggest that much of the material excreted from the blood is not passed out of the body, but is removed to the hypodermis, deposited in the cuticle, and cast off with the exuvium, or in adult aphids becomes accumulated there. Accumulation of polonium over long periods is suggested by Exps. XIII and XIV, Table II, and can also be observed in the results given in Table V. Aphids fed with dyes also show accumulation in the exuvia. Those of eosin-fed aphids are nearly always bright pink even when no eosin can be detected in the gut. These facts are of interest in view of the hypothesis that infection may be caused in plants by aphids excreting sufficient virus on to the leaf to cause infection, if deposited on damaged places, or those which subsequently become damaged. From the figures obtained in those experiments the amount deposited by one aphid in a period of 12-24 hours would be 0.00019 c.mm., and at present dilution experiments with mechanical infection seem to indicate that it would take a much larger amount to give the percentage of infection which is actually obtained with small numbers of aphids. This refers to Hy. III, and not to the viruses which give infection at very high dilutions.

(2) *Relations of volumes imbibed by artificial feeding to those obtaining in natural conditions.*

Several attempts were made to find out by some other means the volume of liquid which could be imbibed by *Myzus persicae* under natural conditions, and the only one which gave consistent results was the

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weighing method referred to above. This, in spite of its obvious disadvantages, gave a consistent figure and one which is reasonably comparable with the radium results.

Table V.

Volumes of liquid imbibed by Myzus persicae determined by weighing.

	Original weight gm.	Weight after starving gm.	Weight after feeding 6 hours gm.	Gain gm.
<i>Natural feeding.</i>				
Weight of	0.0570	0.044	0.0515	0.0075
100 aphids	0.0434	0.0368	0.0444	0.0076
	0.0565	0.0432	0.0521	0.0091
	0.0583	0.0396	0.0469	0.0073
<i>Artificial feeding.</i>				
Weight of	—	0.0311	0.0283	-0.0028
100 aphids	—	0.0394	0.0410	+0.0016
	—	0.0248	0.0261	+0.0013
	—	0.0342	0.0260	-0.0082
	—	0.0278	0.0286	+0.0008
	—	0.0320	0.0349	+0.0029

Table V gives the actual weights recorded. The first part, the results from leaf feeding, shows the gain to be from 0.007 to 0.009 gm., representing 7-9 c.mm. of water or about seven times the average volume obtained by the radium method, which is 1.05 c.mm.

The second part of the table shows the figures obtained from artificial feeding. It can be seen that in some cases there is no gain at all but actually a loss. This would account for those radium experiments, which fail to give any result, and the vital stain experiments in which only about six aphids out of 100 feed. The rest of the 100 would go on losing weight, and any gain by the six would be completely masked. In the same way there is probably a good deal of masking, even in the positive results which may explain their smallness compared with the natural feeding figures. They do, however, compare very favourably with the radium figures, having a range of from 0.8 to 2.9 c.mm. as compared with 0.27-2.6 c.mm. for the radium results.

These results indicate that, when allowance is made for the smaller proportion of aphids feeding, the values obtained for the volume of solution imbibed in the polonium-agar feeding experiments are comparable with those found in feeding in natural conditions. To this extent it is justifiable to assume that the polonium solution will behave in a similar manner to plant juice, when imbibed by the aphid.

(3) *The volume of liquid returned to the leaf by the aphids.*

The results of those experiments in which the aphids were removed from the capsules and placed on *Hyoscyamus* leaves to feed are given in Table VI. In calculating the volume of liquid passed into the leaves it has to be assumed that the concentration of polonium in the solution is the same as that in the original solution.

Table VI.

		Time fed hours	N	d	Q	D	c.mm.	% ejected
XV	Control	12	40	0.75	0.02	70	Imbided 0.53	6.7
	Leaf	24	100	0.125	0.02	70	Ejected 0.35	
XVI	Control	24	80	9.75	0.05	222	Imbided 2.6	5.7
	Leaf	24	80	0.5	0.05	222	Ejected 0.15	
XVII	Control	12	60	1.79	0.01	23	Imbided 1.29	10
	Leaf	24	80	0.25	0.01	23	Ejected 0.13	
XIX	Control	12	300	1.173	0.01	60	Imbided 0.65	8.4
	Leaf	24	275	0.92	0.01	50	Ejected 0.055	
XX	Control	18	44	0.77	0.01	50	Imbided 0.34	13.2
	Leaf	24	98	0.22	0.01	50	Ejected 0.045	
XXI	Control	24	100	4.64	0.05	187	Imbided 1.2	7.6
	Leaf	48	100	0.3	0.05	187	Ejected 0.09	
XXII	Control	12	130	2.7	0.01	74	Imbided 0.26	8.1
	Leaf	12	130	0.2	0.01	74	Ejected 0.021	
XXIV	First feeding	12	40	0.775	0.05	260	Imbided 0.37	5.1
	Leaf feeding	12	100	0.26	0.05	260	Ejected 0.019	

In most of these experiments the preliminary feeding period was 12 hours. The time was fixed to bring the experiments into line with a series of aphid infections on growing plants which were being carried out at the same time. Such a period was sufficient to give a high percentage of infection, under optimum conditions. In both series of experiments the preliminary feeding took place overnight from 9.30 P.M. to 9.30 A.M. In the radium experiment this was followed by a period of leaf feeding from 9.30 A.M. till either 9.30 P.M. or 9.30 A.M. on the following day. As in the previous experiments there was a number of failures, and the amounts imbided by the controls varied considerably.

The way in which the control aphids were taken has already been described. They generally involved smaller numbers than were transferred to the leaves, unless a very large number were fed, because it was necessary to have a large number of aphids so as to increase as far as possible the amount of polonium put into the leaf.

The volume of liquid returned to the leaf again shows wide variation, but this variation is entirely accounted for by the variation in the quantity of liquid imbided.

The results are plotted in Fig. 1, in which a straight line passing

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through the origin has been fitted to the points. The regression coefficient of volumes of liquid transferred on volume imbibed by controls is 0.069 with a standard error of ± 0.0065 , so that the regression is very highly significant. The fitting accounts for 94 per cent. of the variance of the volume of liquid transferred. These results are to be interpreted as showing that a constant fraction (6.9 ± 0.0065 per cent.) of the solution imbibed is transmitted by the insect to the leaf.

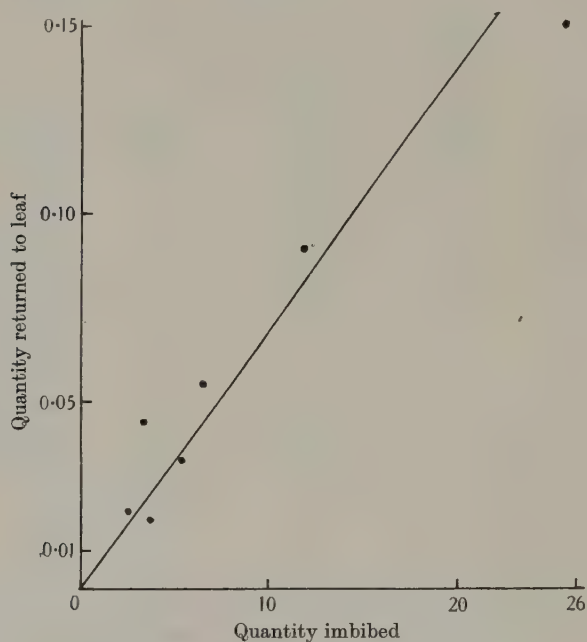


Fig. 1. Graph showing relation between quantity of radium solution imbibed and quantity returned to the leaf.

(4) *The role of salivary ejaculation in the transfer of radium from capsule to leaf.*

Although figures have been obtained showing the volumes of liquid imbibed by the aphids, and the amount found in the leaf after their second feeding, there may still be some doubt as to whether this second volume of liquid is actually passed through the body of the aphid or whether there is still a possibility that it is delivered by the leaf as a coating on the outsides of the stylets. However, the results given in Table VI and Fig. 1 are a convincing argument that the direct method is not a possibility.

It has already been said that a large percentage of aphids actually penetrate the feeding membrane (cf. p. 251). In doing this they form a

coating or tube of salivary material round the stylets which in itself protects them from receiving a deposit of radium. When the stylet is withdrawn these tubes remain and a great many of them can be seen on a membrane on which aphids have been feeding (they stain strongly with eosin: Davidson(2)). Thus it is obvious that whatever the amounts imbibed, and the percentage of aphids which imbibe sufficient to be detected, they have all an equal chance of picking up radium on the outsides of the stylets if that were possible. If they did this then the points shown on Fig. 1 would be grouped about a straight horizontal line, and such a close relation existing between them as is demonstrated by these figures is extremely improbable. Therefore it seems to be established that in order to reach the leaf the polonium solution must pass through the bodies of the aphids. As far as is known the only possible route is through the alimentary canal wall into the blood stream, and from thence to the salivary glands to be ejected by them down the ejaculatory passage in the stylets.

DISCUSSION.

From these results there is good evidence to suppose that up to the end of the first aphid feeding the polonium solution behaves in the same way as the plant juice, since it has been shown that both are taken up by the aphids in similar volumes. That the method and quantitative relations of transmission from the aphid into a leaf are similar for polonium and virus particles is more difficult to demonstrate, but there is some evidence to justify this. Firstly, there is the observed fact that both polonium and virus can be transmitted to a leaf by means of *Myzus persicae*.

Secondly, a constant proportion of the polonium imbibed by the aphids is transmitted, and a similar relation must exist in the transmission of virus, since it has been found in the series of plant infection with Hy. III referred to on p. 247 that the percentage of plants infected by a given number of aphids is constant, if allowance is made for variations in external conditions and source of infection.

Thirdly, it has been argued, from the constancy of relations between amount of polonium imbibed and the amount transmitted, that it is passed through the bodies of the aphids and not transmitted on the outsides of the stylets, and the observed failure to transmit virus by artificial feeding suggests that this is also true for the virus. The theory that the presence of the salivary sheath prevents transmission on the outsides of the stylets would hold good equally for virus and polonium whether in the plant or in artificial media. This suggests an explanation for the fact (which has for so long been difficult to understand for juice-

transmitted viruses) that not every species of aphid which feeds upon an infected plant transmits the virus. It is not unreasonable to suppose that the virus can only survive the action of digestive juices and excretory mechanisms in a limited number of species.

Finally, it should be pointed out that no evidence has been found which conflicts with the hypothesis that the mechanisms of transmission for virus and polonium are essentially the same.

SUMMARY.

1. A method is described for the feeding of *Myzus persicae* on media of which one constituent is a radioactive indicator containing polonium.

2. The results show that *Myzus* picks up the indicator from the medium and transmits it to a leaf on which it is subsequently fed.

3. The volumes imbibed are of the same order of magnitude as those imbibed by aphids under natural feeding conditions.

4. A constant proportion of the amount imbibed is transferred to the leaf, from which it is deduced that the polonium is transmitted through the bodies of the aphids and not on the outsides of the stylets.

5. Evidence is given to show that the virus probably behaves in the same way as the polonium.

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NOTES ON THE TIMOTHY GRASS FLIES (*AMAUROSOMA* SPP.)

BY H. F. BARNES, M.A., PH.D.

(*Entomology Department, Rothamsted Experimental Station.*)

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I. FOREWORD.

IN view of the fact that Mr L. A. L. King, Miss Agnes A. Meikle and Mr A. Broadfoot have recently been investigating the Timothy grass flies⁽⁸⁾ in Scotland, it seems desirable to place on record some information regarding these flies obtained in the south of England, incomplete as it admittedly is.

In July 1931 Mr D. J. Columbus Jones of Messrs Sutton and Sons, Ltd., Reading and Slough, complained to the writer of damage being done to the seed heads of pedigree selections of Timothy grass, remarking that he could find nothing attacking the heads directly. However, on unfurling the flag-leaf sheath he found in each case a small pupa. He further stated that the damage appeared to be done before the heads came out of their respective sheaths. This was a typical attack of the Timothy flies and steps were taken to start an investigation as to the species involved, their bionomics and parasites and the incidence of attack on early and late strains of the grass as well as the effect of manuring.

Owing to the pressure of other work the investigation was allowed to lapse when it was realised that Mr King and Miss Meikle were undertaking a similar study⁽⁷⁾. These notes are therefore incomplete. More recently we have got into touch with these workers and exchanged our information. As a result an understanding has been reached whereby

their paper and these notes appear simultaneously and no overlap has been allowed to occur.

For the sake of other workers a brief historical review of these pests has been added to these notes.

II. IDENTIFICATION AND HISTORY.

The Timothy grass flies (*Amaurosoma* spp.) were until recently placed in the family Cordyluridae of the Diptera. Curran⁽³⁾, however, now includes them in the family Muscidae, in which family he also places the Scatophagidae, Anthomyidae and those Muscidae lacking hypopleural bristles.

The two species known to attack Timothy grass are *Amaurosoma armillatum* Zett. and *A. flavipes* Fall.

A. armillatum was described in 1846⁽²⁴⁾ as a *Cordylura* and placed by Becker in the genus *Amaurosoma* in 1894⁽¹⁾.

A. flavipes was described in 1819⁽⁴⁾ also as a *Cordylura*. Schiner in 1864 mentions it as a *Cleigastra*⁽¹⁹⁾ and Becker made it the type of his newly described genus *Amaurosoma* in 1894⁽¹⁾.

Among the earlier writers on these flies, Nowicki⁽¹⁵⁾ mentioned *A. flavipes* as a pest of Timothy grass in Galicia in 1873; while Taschenberg⁽²²⁾ dealt with this species in Germany in 1880 and Lindeman⁽¹¹⁾ worked out its life history in Russia in 1887. Scandinavian entomologists, including Lampa and Schøyen, investigated the bionomics of both species about the beginning of the century⁽¹⁶⁾. Since then short paragraphs concerning them have appeared in such text-books as Sorauer's *Handbuch der Pflanzenkrankheiten*⁽²¹⁾ and Rostrup and Thomsen's *Vort Landbrugs Skadedyr*⁽¹⁷⁾ and the German translation of this work, *Die tierischen Schädlinge des Ackerbaues*⁽¹⁸⁾.

Among the more recent papers dealing with or mentioning these flies the following are the more important. Schøyen⁽²⁰⁾ advised deep ploughing in the autumn to bury the puparia or the omission of Timothy grass from the rotation for a year or two. Korff⁽⁹⁾ suggested that the abundance of *A. flavipes* was due to premature warmth in the spring and drought in the early summer. He was doubtful as to the number of generations of the fly in the year. Wahl⁽²³⁾ described the life history and stated that the seed crop of Timothy grass failed owing to attacks by *A. flavipes* and *A. armillatum*. Kreiter⁽¹⁰⁾, in a description of the morphology and biology of Diptera occurring in grasses, included *A. flavipes* in a key to the larvae and pupae. Lastly, Karpova⁽⁶⁾, whose paper perhaps gives more information than any of the others, states that the two species are

almost indistinguishable in the immature stages. He says that the eggs are laid on the upper surface of the leaves of winter rye and Timothy grass by the end of May or, in unfavourable seasons, mid-June. The larval stage lasts 15–17 days mostly in the sheaths of the leaves. The larvae migrate to the ears when these are fully developed. Pupation occurs in the soil where the puparia remain over winter. Laboratory work showed that the feeding of the larvae decreases the weight and length of the ears and prevents the normal growth of the uppermost internode of the stem, especially in rye. He calculated that the ultimate loss in 1928 and 1929 in the Leningrad and Smolensk Governments to the seed crop of winter rye and Timothy grass was 5 and 10 per cent. respectively.

Besides these papers there are several records of Timothy flies doing damage. For example, they have been reported from Denmark, Sweden, Norway and Russia several times and also from Germany and Finland.

Though these flies occur in Great Britain and their damage has been familiar to the writer and other workers for several years, there appear to be singularly few published records of their occurrence in this country. MacDougall⁽¹²⁾ figured the damage and stated that it was due to a fly maggot, but that he had failed to rear the fly. Newton⁽¹⁴⁾ reported the occurrence of *Amaurosoma* sp. on the farm at Rothamsted in 1931. Recently King and Meikle⁽⁷⁾ stated that they had reared *A. armillatum* Zett. and gave a preliminary account of its life history.

Timothy grass (*Phleum pratense*) is the usual host plant but winter rye has been mentioned at least three times by Russian workers. It is an open question whether this is an instance of erroneous identification.

III. DISTRIBUTION.

A. flavipes has actually been recorded from Norway, Sweden, Denmark, Germany, Austria (Vienna) and Russia. *A. armillatum* has been reported from Scotland, Sweden, Austria (Vienna) and Russia. In addition *Amaurosoma* sp. has been recorded from Finland. Actually these specific reports are not necessarily of much value, owing to the fact that the damage is so similar in both species, and therefore unless the flies are reared there is great possibility of wrong determination. They do show, however, the general distribution of the Timothy flies.

The present writer has reared both species from material obtained near Slough, England, and Ieuan Thomas has seen damage by one or both of these species at Aberystwyth. *Amaurosoma* sp. has also occurred on the farm at Rothamsted. Mr J. E. Collin, who has kindly determined both species, writes (31. v. 32) that while he has actually never taken

A. flavipes he has seen several British specimens, and also that *A. armillatum* appears to be the more common of the two, though not so common as *A. tibiella* Zett. and *A. fasciata* Mg. It would be of interest to know the life histories of these two other species which were on the British list at least as early as 1899⁽¹³⁾.

There are also six species belonging to this genus occurring in Canada (2), but so far their life histories are unknown.

IV. BIONOMICS.

Puparia collected at Reading, Berkshire, kept over winter in an outdoor insectary at Harpenden, Hertfordshire, started producing flies in 1932 during May. Similar material in 1933 started emerging on April 11th, but this was an "early" year. In this latter year the last flies to emerge appeared on May 4th. *A. armillatum* seemed to emerge slightly in advance of *A. flavipes*. Reuter's⁽¹⁶⁾ dates and figures, however, seem to indicate the reverse, but both his and the writer's numbers are small.

From rough experiments it would appear that the flies can live at least ten days. In one case a male fly caught and sucked a gall midge adult female, *Rhabdophaga triandraperda* Barnes, but usually they seemed to enjoy feeding on a water solution of sugar which was placed on cotton-wool in the rearing cages. About twice as many *A. armillatum* adults have been bred as *A. flavipes*, thus helping to confirm Mr Collin's view that *A. armillatum* is the more common species. Reuter⁽¹⁶⁾ reared twenty *A. flavipes* and thirty-two *A. armillatum*.

The eggs are certainly usually laid on the upper surface of the blades of grass, and although it is the rule to find only one egg per blade, on occasion as many as three have been found. Quite often in the field plants of Timothy have been seen bearing eggs on more than one leaf blade.

As soon as the larvae hatch they work their way down to the developing ear. Their exact progress has not been observed, but it has been noted that when they approach the ear, which at this stage may be only about 1 cm. or less in length, they bore straight through the enveloping sheaths. Having reached the ear they feed on it and remain on it as it grows upwards. In this way they get carried up as far as the flag-leaf sheath. By this time the larvae are fully grown or nearly so. The ear of grass goes on growing bearing the characteristic signs of attack as soon as it breaks the sheath (and actually long before). In no case have larvae been seen feeding on the ear after it has finally burst from the flag-leaf sheath, either by day or night.

Puparia have been found in the flag-leaf sheath on several occasions, though it is considered probable that pupation usually does occur in the soil. As the grass dries up the puparia must either fall out of the flag-leaf sheaths or get carried to the ground with the dying grass. Puparia were first found in 1932 on June 24th.

As an indication of the rate of larval development the following information is given. In samples of infested grass dated 2. vi. 32, although most of the larvae were very small and chiefly in the first instar, a few were about half-grown, these larger larvae being on an early variety of Timothy grass. By 8. vi. 32 most of the larvae were half-grown, but a sample of a late variety of grass provided some first-instar larvae. By 24. vi. 32 nearly all the larvae were full grown and puparia were found in the flag-leaf sheaths.

V. PARASITES.

The first record of a parasite seems to be that of Reuter⁽¹⁶⁾ who reared an unidentified *Pteromalid*.

Two parasites have been reared from material received from Slough: *Microbracon exhilarator* Nees and *Lamprotatus* sp. The writer is indebted to Dr Ch. Ferrière for their identification. The *Lamprotatus* sp. appears to be the more common parasite. For example, in 1932 one specimen of each parasite was reared, in 1933 nineteen *Lamprotatus* sp. and two *M. exhilarator* and in 1934 three *Lamprotatus* sp. to one *M. exhilarator*.

The parasites emerged during the first week of June in 1932, in May (11th-27th) in 1933 and between May 5th and June 11th in 1934.

VI. INCIDENCE OF ATTACK.

(i) *Effect of earliness or lateness of Timothy grass strain.*

Through the courtesy of Messrs Sutton and Sons the writer has been able to make some preliminary examinations of samples of different strains of Timothy grass from an infested area.

The results of such examinations made in 1932 and 1933 are given in Table I. Mr Columbus Jones has kindly arranged the strains in their order of flowering.

In 1933 additional strains were examined, and the percentage infestations on June 22nd are shown in Table II.

It will be seen that the incidence of attack in 1932 was severe and decidedly greater than in 1933. The plants in 1933 were in a different area, and this moving of the plants to a fresh position may account for the lowering of the attack. These two years were bad ones in which to

Table I.

*Comparative infestation of different strains of
Timothy grass, 1932 and 1933.*

Strain	Order of flowering	Percentage infestation			
		1932			1933
		June 4th	June 24th	July 2nd	June 22nd
Sutton's 36	1 early	50	25	—	23
35	2	80	63	—	60
34	3	80	—	—	38
37	4	60	94	100	34
33	5 late	70	—	—	35
	Average	68	61	—	38

Table II.

*Comparative infestation of additional strains of
Timothy grass, June 22nd, 1933.*

Strain	Order of flowering	Percentage infestation
Stapledon's Pasture Type (S 49)	1 early	15
Sutton's Dwarf	2	9
Canadian	3	44
Scotch	4	33
Swedish "Gloria"	5	44
Swedish "Primus"	6	22
Sutton's Pedigree Leafy	7 late	41

study the problem, as in one case the spring was exceptionally early. However, there is some indication that the earliest strains are less liable to attack in an early season. It does not, however, appear feasible to get limits wide enough to cover the period of attack. There may be some varietal resistance in addition, but so far there has been no indication of any appreciable resistance or immunity, with the possible exception of strain 36 which is a dwarf variety. This is supported by the low figure of Sutton's Dwarf.

(ii) *Effect of manuring.*

In view of Frew's results⁽⁵⁾ with the gout fly and the similarity between the position of the eggs and the habits of the larvae of this fly and those of the Timothy flies, it was thought that manuring the grass might be helpful in reducing attack. Strains of grass which were unmanured (35 and 36) and manured (56.21 and 56.20, the counterparts of 35 and 36) were therefore sampled. The results of this enquiry are given in Table III.

Table III.
Effect of manuring.

Strain	Unmanured percentage attack		Strain	Manured percentage attack	
	4. vi. 32	24. vi. 32		4. vi. 32	24. vi. 32
35	80	63	56.21	35	68
36	50	25	56.20	24	21

It would appear from these figures, though few, that manuring may have some beneficial effect in reducing infestation by the larvae of these flies. Further investigations, however, will have to be made before one can be certain of this. The effect of manuring may only be an initial one at the beginning of the season, but even this would be helpful.

It is interesting to note in connection with the above figures that the incidence of attack in Aberystwyth on July 1st, 1932, was nil on strain Bd 310 and only 2-3 per cent. on Ordinary Commercial.

VII. SUMMARY.

1. A short account of the systematic position of the Timothy flies (*Amaurossoma armillatum* Zett. and *A. flavipes* Fall.) is given and the literature concerning them is reviewed.

2. Their distribution is stated and notes are given on their bionomics as observed at Harpenden on material obtained from Slough, Buckinghamshire.

3. The parasites *Microbracon exhilarator* Nees and *Lamprotatus* sp. are recorded.

4. Efforts have been made to see whether the earliness or lateness of Timothy grass strain has any effect on the incidence of attack. There are indications that a dwarf strain which is an early one is less liable to attack, though this may be due to a varietal resistance rather than to the time of flowering.

5. It does not appear feasible to get limits of flowering wide enough to cover the period of attack, so that further investigations in this direction need not be pursued.

6. Indications were obtained that the effect of manuring the grass may be to lower the attack, but further work in this direction is required.

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OBSERVATIONS ON THE TIMOTHY GRASS FLY (*AMAUROSOMA ARMILLATUM* ZETT.)

BY L. A. L. KING, M.A., AGNES A. MEIKLE, N.D.A.

(*West of Scotland Agricultural College, Glasgow*)

AND

A. BROADFOOT, B.Sc., N.D.A.

(*Extension Staff*).

(With 2 Text-figures.)

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I. INTRODUCTION.

DAMAGE caused to Timothy grass (*Phleum pratense*) by the larvae of this fly has been recorded, in the course of the last twenty years, from Sweden, Austria, Russia and Scotland (1). It consists of a partial stripping of the flower-heads before these have emerged from the sheath. This stripping may occur at the apex of the head, in the form of a girdle lower down or as a track, straight or curved, passing down one side of the inflorescence. The buds are cut off leaving a succession of scars. Heads of Timothy grass showing injury of this type have been received from the counties of Stirling, Perth, Renfrew, Ayr and Lanark, from the islands of Skye and Barra, and from the north coast of County Antrim in Ireland.

The attack is recognised as serious where the crop is a perennial one and the grass is grown for seed. In the carse areas of Stirling and Perth the loss to growers has been estimated at from 25 to 30 per cent. of the seed.

Damaged heads containing the larvae were brought to the writers at the end of June in 1931. The stalks were inserted into damp sand at the bottom of glass tubes which were then plugged with cotton-wool. Puparia were obtained and were kept indoors in the sand until the following April when flies emerged. From these the species was determined, and in June 1932 flies of the same species were caught in the field and eggs were found there on the plants. Flies brought back alive were placed with egg-free plants in glass breeding cylinders and oviposition took place. Hatching was observed and the mode of entry of the larva noted. Larvae at various stages of growth were examined and puparia were collected in the field after the grass had "shot", *i.e.* the flower-heads had emerged from the sheath. It was apparent that there was only one generation of flies in the year.

As the season of activity of the flies was found to be short an attempt was made, early in 1933, to hasten, by artificial fertilisers, the "shooting" of the plants so as to derange the time-incidence of the fly. The results were unpromising. Another line of attack was tried in the use of deterrents, sprays and dusts, at the egg-laying season in 1933. The experiment was vitiated by the unexpectedly early oviposition in that year, so further trial of this method was made in May 1934. Two of the substances used seemed to give a measure of protection, and the experience suggests the possibility of limiting the incidence of the attack upon small areas selected for the production of seed.

II. BIONOMICS.

In Stirlingshire and West Perth the flies appear about the middle of May and remain on the wing up to the end of the first week of June. The earliest date at which we have seen them in the field is May 11th (1934) and the latest June 8th (1932). They are on the wing for some days before egg-laying commences. About twice as many males as females are captured by a net used in "sweeping" the grass.

The earliest date on which eggs were found was May 15th in 1933, and May 23rd in 1934. The eggs are laid at the base of a leaf, just above the ligule and roughly parallel to the veins. In most cases a plant showed only one egg, but, where infestation was severe, several might be found on the same plant and, exceptionally, two eggs occurred on a single leaf.

The earliest date on which larvae were detected was May 23rd in 1933. In 1934 they were not found until May 28th. On May 15th, 1933, a plant with two eggs on one leaf was transferred to a pot and brought into the laboratory. These eggs hatched on May 23rd, showing viability after at least 8 days from oviposition. In the laboratory, in both 1932 and 1934, eggs laid by captive flies hatched in from 4 to 6 days. The process of hatching may be a lengthy one, as, in a single instance observed, it began about 10 A.M. and was not completed until 10.30 P.M. the same day.

The newly hatched larva perforates the leaves immediately surrounding the inflorescence if these are still tightly rolled at the time. If they have begun to unroll it makes its way in without leaving any mark of its presence. The damage to the flower-head is completed before the head appears.

The larval period lasts about 15 days in the laboratory and three larval instars are recognised. The fully fed larva leaves the plant, buries itself in the ground at a depth of 1-2 in., and is gradually transformed into a puparium. This transformation may be delayed as long as 14 days, but is usually completed in 3 or 4 days.

The puparium remains, apparently unchanged, throughout the winter and, even in a heated laboratory, emergence does not take place until the following spring. There is no indication of a second generation in the year.

III. PARASITES.

From one of the puparia obtained in 1931 there emerged, about April 20th, 1932, a Pteromalid Hymenopteron, *Seladerma laetum* Walk. Of thirty puparia of *Amaurosoma*, kept through the winter 1933-4, thirteen yielded each a single specimen of this parasite. This indicates a high rate of parasitisation. The parasites emerged at various dates between the end of April and the middle of June, but most of them in the latter month. Two specimens of the Braconid Hymenopteron *Dacnusa semirugosa* Hal. emerged at the end of the first week of June 1934 from puparia of a batch which yielded *Seladerma laetum* from other individuals.

IV. DESCRIPTIVE NOTES.

The early stages of *Amaurosoma armillatum* agree in general structure with those of other Diptera Cyclorrhapha, e.g. as described by Thomas (5). Accordingly only brief descriptions are given below.

(a) *The egg.*

Measurements in length vary from 1.0 to 1.17 mm., in breadth from 0.23 to 0.34 mm. The colour at first is very pale yellow, but darkens, before hatching, to a wheat-straw tint. The shape is boat-like, tapering towards both ends. The lower surface is markedly convex and seems to be sticky at the time of laying. The upper surface shows an inward fold along each side, and this may be accompanied by other ridges, apparently due to shrinkage, before the time of hatching. The whole surface of the egg has an areolate sculpture which is obscured on the under surface by the sticky substance referred to above. Hatching begins at the micropylar end, which is usually the one directed away from the ligule of the leaf.

(b) *The first-instar larva.*

The smallest example of this stage examined measured 1.06 mm. in length. The larva is almost transparent, with a black cephalo-pharyngeal

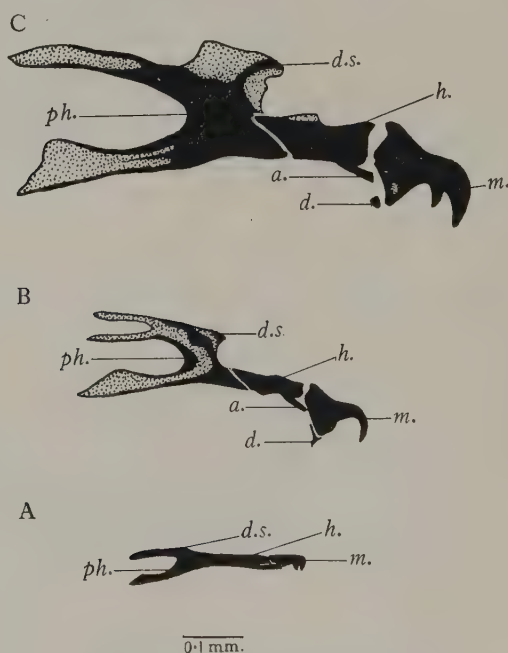


Fig. 1. Cephalo-pharyngeal skeletons of the larval instars of *A. armillatum*. A, first-instar larva; B, second-instar larva; C, third-instar larva—lateral view. *m.* mandibular sclerite; *d.* dentate sclerite; *a.* accessory sclerite; *h.* hypostomal sclerite; *d.s.* median dorsal sclerite; *ph.* pharyngeal sclerite.

skeleton extending through one-quarter of the total length. The *mandibular sclerites* are longer, in proportion to their height, than those of succeeding stages, and each ends in two blunt down-curved teeth of approximately equal size, one in front of the other and very nearly in the same vertical plane. The *hypostomal sclerite* is not separate from the *pharyngeal sclerite* (Fig. 1 A). Above the mouth, on each side, is a transverse row of twelve to fifteen stout, blunt denticles. Above these are the *maxillary palps* and above these again the short two-jointed *antennae*.

The larva is metapneustic and each of the *posterior spiracles*, which are borne on cylindrical projections, has two oval apertures.

The *integument* of all the segments is smooth except on the anterior margins. These are traversed by several rows of extremely minute denticles.

(c) *The second-instar larva.*

The smallest of this stage examined measured 2.5 mm. in length. The cephalo-pharyngeal skeleton occupied one-fifth of the total length.

The *mandibular sclerites* are sickle-shaped, each ending in a single claw-like tooth behind which is an inconspicuous mound. *Dentate sclerite*, *hypostomal sclerite*, *accessory sclerites* and *pharyngeal sclerite* are distinct (Fig. 1 B).

The larva is now amphipneustic. Each *prothoracic spiracle* bifurcates widely and bears twelve to fifteen short, rounded *digitate processes* (Fig. 2 A). Each of the *post-abdominal spiracles* opens by two broadly oval slits.

The *integument* bears several rows of denticles on the anterior ventral margin of the prothorax and of the succeeding segments, in addition to scattered groups, in transverse rows, some reaching the dorsal surface, others occurring in intermediate positions on the ventral surface.

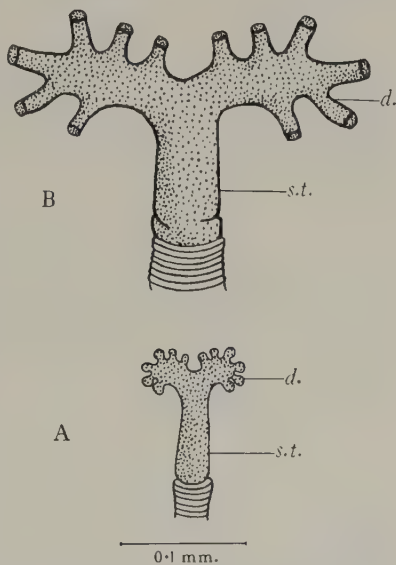


Fig. 2. Anterior spiracles of the larvae of *A. armillatum*. A, second-instar larva; B, third-instar larva. *s.t.* stigmatic trunk; *d.* digitate process.

(d) The third-instar larva.

Specimens of this stage measured from 4.2 to 6.8 mm. in length and up to 2.0 mm. in breadth. The cephalo-pharyngeal skeleton extends through one-sixth to one-eighth of the total length.

Each *mandibular sclerite* has a strong apical tooth and one, not much less strong, behind it. These teeth are stouter than the single tooth of the second-instar (Fig. 1C).

Either of the *prothoracic spiracles* consists of a *stigmatic trunk* which divides into two branches, each carrying six to seven *digitate processes* (Fig. 2B). In some cases an additional process is found at the junction of the two branches. The digitate processes are longer and less rounded than in the previous instar and each has a chitinous ring at its distal end. Each *post-abdominal spiracle* consists of a stigmatic trunk which divides into three, a short distance below the *stigmatic plate*. These open on the stigmatic plate by three elongated oval slits, each surrounded by a chitinous border. The stigmatic plate also is bordered by a chitinous ring.

The *integument* has its denticles more strongly developed, especially on the anterior margins of the thoracic segments and on the anterior and posterior margins of the abdominal segments. Their numbers and arrangement show considerable variation. The *post-anal pseudopods*, on the ventral surface of the last abdominal segment, are armed with several rows of denticles.

(e) The puparium.

The lengths of specimens varied from 4.0 to 5.1 mm. and the breadths from 1.5 to 2.0 mm. The colour was yellow at first, gradually changing to a reddish brown. The form of the spiracles of the late larva is still discernible in this stage.

(f) The imagines.

The *male* is approximately 4.5 mm. in length and has a wing spread of about 7 mm. The *female* is about 5.5 mm. long, with a wing spread of 9 mm. Both sexes appear dark grey in colour except for the maxillary palps, the distal extremities of the femora, the whole of the tibiae and the halteres, which are reddish yellow to reddish brown, and the antennae which are black. The coloration of the legs is a feature useful in the field for the separation of these from other flies of similar size.

V. EXPERIMENTS ON CONTROL.

Deep ploughing in autumn has been recommended by Schøyen (4) and by Korff (2) for attacks by an allied species (*Amaurosoma flavipes* Fall.) of similar habits. This procedure appears unsuited to the heavy carse-land of the district under examination. It is undesirable also because Timothy grass is here grown as practically a perennial crop for hay and only a portion, chosen by its appearance after flowering, is left standing for seed.

Since the puparia of *A. armillatum* lie close to the surface of the ground it appears reasonable to suggest such measures as (i) running poultry on the land shortly after the cutting of the final crop, (ii) heavy rolling of the land during the dormant season to disturb or crush the puparia. These measures await experimental investigation. As alternatives to the above we have tested in the field (iii) *the application of phosphatic fertilisers* to hasten the emergence of the inflorescence, since the "shot" head is free from further attack, (iv) *the application of "deterrent" substances* during the short egg-laying season of the flies to protect the plants from attack.

Phosphatic fertilisers.

On each of five farms, three in west Perthshire and two in Stirlingshire, a number of experimental plots was marked out, each measuring one-eighth of an acre. These were on land already carrying Timothy grass of various ages, from 2 years up to 15 years.

On February 16th or 17th, 1933, one plot on each farm received the heavy dressing of 2 cwt. of superphosphate, another plot 2 cwt. of basic slag. On these plots and on the rest of the land, with the exception of certain plots referred to below, the farmers added, in the first week of April, their usual dressings of sulphate of ammonia, $3\frac{1}{2}$ cwt. *per acre* or more.

The special plots referred to above as exceptions received only the phosphates, the sulphate of ammonia being withheld.

During an inspection of the plots on June 3rd, some days after oviposition had begun, random sampling of the plants on one group of plots gave a rough estimate of the distribution of the eggs as follows:

	Plants with eggs %
Basic slag with sulphate of ammonia	30
Superphosphate with sulphate of ammonia	20
Basic slag without sulphate of ammonia	15.7
Control, untreated except with sulphate of ammonia	24

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On the plot *without sulphate of ammonia* "shooting" had already begun but growth did not exceed 2 ft. in height, the plants were pale in colour and there was a marked development of weeds such as *Equisetum*, dock and Yorkshire fog. On the plots *with sulphate of ammonia*, as on the controls, shooting was retarded but growth had reached a height of 2 ft. 8 in., the colour was green and there was little development of weeds. No marked difference could be detected by the eye between the phosphate-treated grass and that which had received sulphate of ammonia alone.

On subsequent dates estimates were made, on this and three of the other farms, of the *percentage of damaged heads* showing the particular type of injury associated with *Amaurosome*. The fifth farm (c), on which the Timothy grass was only 2 years old, showed only a few eggs and is not taken into account.

The results may be summarised as follows:

Farm	Percentage of damaged heads			
				(a)	(b)	(c)	(d)
Control				37	56	50	24
Basic slag with sulphate of ammonia				35	62.7	55.3	14
Superphosphate with sulphate of ammonia				30	56	59	24
Basic slag alone				22.2	(—)*	39.3	10

* In the case of farm (b) the appearance of the growth, due to the absence of the sulphate of ammonia, caused the farmer to depart from the instructions and to add the usual dressing before our estimate was made. The result was that the figure for the plot rose to 60.

It will be noted that the only definite conclusion suggested by these figures is that the addition of sulphate of ammonia increases the liability to attack. This was confirmed, during the past year (1934), by the observation that in two adjoining fields, one of which had received a heavy dressing (5 cwt. per acre) of sulphate of ammonia and the other a light one (2½ cwt.), the rates of infestation were different, viz.

Heavy dressing	Eggs on 32 % of the plants
			Damaged heads 24.7 %
Light dressing	Eggs on 20 %
			Damaged heads 15.9 %

As we have indicated above the *omission* of the ammonia salt has detrimental effects. The length of the flower-heads produced was found to be only 1½ in. as against 3 in. in the fully dressed plants. A strict *limitation* of the amount of this dressing seems to be a desideratum in combating the fly.

Deterrents.

On May 19th, 1933, preliminary experiments were made on two of the farms in west Perthshire with sprays, viz. an undiluted paraffin oil, an emulsion of crude naphthalene with paraffin and soft soap, and a similar emulsion of flake naphthalene; also with a dust of lime with crude creosote. It was found that egg-laying had already begun, so that *prevention* could not be tested.

Subsequent counts (on July 5th) of damaged heads gave the following figures:

Farm	(a)	(b)
Control			36.3	42
Paraffin alone			36.0	39
Crude naphthalene emulsion			27.0	36
Flake naphthalene emulsion			31.0	36
Lime-creosote dust			27.0	22

The apparent decrease in attack where these substances had been used seemed to justify a further trial. Accordingly, on May 11th, 1934, experimental strips were marked out on the farm referred to above as farm (a) and on an adjoining farm, referred to below as farm (f). Each strip was 25 yd. long and 2 yd. wide. On farm (a) the strips were separated by intervals of 2 yd., on farm (f) by untreated spaces 5 yd. wide. Each strip was treated with one of the following substances:

(i) *Lime-creosote dust* ($3\frac{1}{2}$ lb.) made by mixing finely powdered lime, of a commercial brand, with 2 per cent. of its weight of crude creosote.

(ii) *A proprietary spray fluid* containing about 3 per cent. of tar acids and diluted in the proportion of 1 part of fluid to 80 of water.

(iii) *A spray fluid* (3 gallons) of *crude creosote in ground-nut oil emulsified with ammonium oleate*.

(iv) *A spray fluid* (3 gallons) of *crude naphthalene* similarly prepared.

On this day a few flies, males preponderating, were taken among the plants but no eggs were found. On May 23rd oviposition had begun and a few eggs were found on the untreated and on some of the treated plots. Egg counts were made on farm (f), first on May 26th and again on June 1st. These were followed by a count of damaged heads on July 2nd, when the plants were up to 3 ft. in height and ripening had begun. Counts were made also on farm (a) on June 1st and on July 2nd.

The percentages of infestation estimated were as follows:

Farm (f).	Plants with eggs		Damaged heads July 2nd
	May 26th	June 1st	
Control	11.0	20.0	17.0
Lime creosote	20.0	20.0	19.0
Proprietary	17.5	36.0	12.0
Oil creosote	3.2	16.0	8.9
Oil naphthalene	2.2	10.0	8.5

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On farm (a) counts were made for comparison on two of these dates:

	Plants with eggs June 1st	Damaged heads July 2nd
Control	35.0	21.5
Lime creosote	30.0	15.0
Proprietary	26.0	10.0
Oil creosote	22.0	10.0
Oil naphthalene	10.0	6.0

It appears that a number of the eggs laid do not succeed in producing infestation; that of the deterrents tested lime-creosote dust has little effect, if any, in protecting the plant from oviposition and is unreliable in reducing infestation of the heads; that the proprietary substance may have some residual effect of that kind; that the two oil mixtures are protective, to a variable extent, when first applied and that the crude naphthalene emulsion is, both in immediate and in residual effect, the more promising of the two.

The composition of the naphthalene emulsion, based upon Mesnil(3), was as follows:

Water	7 oz.
Ammonium oleate	3 oz.
Crude naphthalene	2 oz.
Ground-nut oil	18 oz.

The water was heated, but not to boiling, in a deep can and the oleate stirred into it in small quantities at a time. The naphthalene, previously rubbed down in the oil, was poured into the hot oleate which was then reheated carefully to boiling-point and removed from the flame. The ingredients were mixed with a syringe for 15 min. and stored overnight in a can provided with a well-fitting lid. Before use the quantity indicated above was diluted with water to make up 6 gallons of spray fluid.

On June 1st, as flies were still found on the wing, the plots on farm (f) were sprayed again. No second spraying was given on farm (a). No marked benefit was noted, at the later count, as a result of this second spraying.

It is not claimed that the figures obtained give a precise measurement of the effect of each preparation, since there is some tendency to "patchiness" in distribution of the eggs, but the figures from which conclusions have been drawn were arrived at after comparison of independent counts by two, in some cases by three observers. They seem to justify the recommendation that portions of the fields, to be reserved for seed, should be marked off *early in the season* and sprayed, about the middle of May, with the oil-naphthalene emulsion, that a watch should be kept for the

eggs on the rest of the field, and that, as soon as these appear, the spraying should be repeated on the selected portions. The reason for the double treatment is the uncertainty as to the date on which egg-laying may begin.

VI. SUMMARY.

1. A type of damage to the inflorescence of Timothy grass, which appears to be widely distributed, has been found in the west of Scotland to be due to *Amaurosoma armillatum* Zett.

2. Information is given as to occurrence in the field, method of attack on the plant, life history and characteristics of the insect in its different stages. Three larval instars are distinguished and the life history is noted as monocyclic.

3. Two Hymenopteron parasites are recorded.

4. Experiments are described which were designed to test possible methods of control (a) by soil-treatment, (b) by deterrents.

5. Of the latter an emulsion of crude naphthalene in ground-nut oil is recommended as promising.

6. It is suggested that the grass to be reserved for seed should be set apart early in the season and sprayed with this fluid at a time as near as possible to that of the egg-laying.

VII. ACKNOWLEDGMENTS.

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SHEEP BLOWFLY INVESTIGATIONS

I. THE RELATIONSHIP OF HUMIDITY TO BLOWFLY ATTACK

By W. MALDWYN DAVIES, B.Sc., PH.D.

AND

R. P. HOBSON, B.Sc., PH.D.

(Department of Agricultural Zoology, School of Agriculture,
University College of North Wales, Bangor.)

(With 1 Text-figure.)

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I. INTRODUCTION.

DURING recent years the sheep blowfly problem has been the subject of research in most of the leading sheep-rearing countries of the world. It is, thus, surprising to find, in so great a stock country as Great Britain, that, prior to 1931, this problem was the subject of only one scientific publication (MacDougall(5)). Agricultural writings (Fitzherbert(3)) show clearly that maggot infestation has caused grave concern to the sheep farmer in this country since early times. In North Wales(1) this pest has been in evidence since sheep-farming became an integral part of agricultural practice. Attacks by sheep maggot flies are by no means confined to this province, for a recent publication by Ritchie(7) demon-

strates their importance in Scotland and, in fact, attacks occur in varying degrees wherever sheep-farming is practised in Great Britain. It is fitting, therefore, that the Agricultural Research Council, appreciating the significance of the problem, should come forward with financial assistance. The complex nature of the problem demands that the investigations should not be confined to entomological studies; a preliminary survey in the field by Davies⁽¹⁾ showed clearly the need for collateral research along several lines. One of the writers (R. P. H.) had carried out biochemical investigations on the nutrition of blowfly larvae at the London School of Hygiene and Tropical Medicine; and, in March 1934, arrangements were made under the auspices of the Agricultural Research Council to continue the work, conjointly, at the University College of North Wales, Bangor.

It is particularly desirable that investigations of a fundamental nature should be carried out in this country, since myiasis is confined almost entirely to the activities of the one species of fly, *Lucilia sericata* Mg. The problem, therefore, is not complicated, as it is in the Dominions, by several species of flies being involved. During the last decade, investigations on the sheep blowfly problem have extended from entomological studies solely on the species of blowflies and their life histories, together with empirical methods of treatment, to the wider study of the nature of "strike" and the factors causing susceptibility of the individual sheep. Seddon⁽⁸⁾, working with Merino sheep in Australia, concluded that "sheep are not struck simply by chance but because of the development on the sheep of an area of susceptibility". He was able, in the case of "crutch strike", to group sheep into classes of varying degrees of susceptibility according to the conformation of the breech and the degree of wrinkling of the skin. The problem in Great Britain is complicated by the multitude of breeds and cross-breeds, most of which do not possess the skin folds or wrinkles which Seddon found highly susceptible to fly attack. The predisposing factors need careful study, and this paper is the first of a series dealing with this subject. In order to avoid confusion in terminology, it is proposed to adopt the term "crutch strike" used in Australia for attacks that occur in the region of the breech. Since in this country another common form of myiasis occurs, at least in the early stages, in the region of the shoulder blades, the term "shoulder strike" will be used to differentiate it from the "body strike" of Australia⁽⁴⁾, which includes "cases occurring about any part of the body, apart from the crutch", and which, it appears, may originate quite differently from the "shoulder strike" found in this country.

It is generally recognised that attacks by sheep maggot flies are more frequent in damp, close weather with intermittent bursts of sunshine. However, the exact relationship between the climatic conditions and the incidence of fly attack has not been fully elucidated. Field observations carried out during the dry summers of 1933 and 1934 showed the need for a detailed study of the part played by humidity in sheep myiasis.

II. FIELD OBSERVATIONS.

In 1933 the total rainfall and the mean maximum daily temperatures in the screen during the months when attacks are most common were as follows:

June	1.9 in.	63.6° F.
July	1.66 in.	68.4° F.
August	1.65 in.	71.8° F.
September	1.48 in.	66.2° F.

The temperature during the summer was ideal for the activities of blow-flies, and examination of carrion revealed an abundance of *Lucilia sericata*. The Rhuddland Marsh, on the borders of Denbighshire and Flintshire, which is heavily stocked with sheep, is normally one of the worst districts in the province for sheep maggot attacks. However, in 1933, attacks on flocks totalling over 4000 sheep were negligible, shepherds commenting on the fact that infestations were fewer than in any previous season they could recall. In this and other districts, sheep were observed on which flies had oviposited, but either the eggs or the young larvae (if hatching had taken place) had subsequently dried up. However, in the hill districts of North Wales, where slight rain had fallen at intervals, infestations were severe. The year 1933 was exceptional also in that attacks were common in October and extended even into November; during these two months rain became general and the temperature remained above the normal. In July 1934 drought conditions again prevailed, the total rainfall and mean maximum daily temperature being 1.27 in. and 73.1° F. respectively. Opportunity was taken for a careful examination of seventy-seven Welsh lambs grazing on the lowlands at the College Farm. Two of these lambs had large batches of eggs deposited in the region of the breech; most of the eggs had hatched, but the young larvae remained near the egg batch and were completely desiccated. In two other instances batches of dried-up eggs were found between the shoulder blades. Casual inspection of a flock of lambs on an adjacent farm about the same period revealed four more lambs which had been blown, but most of the eggs, and any larvae which had hatched

were dried up. There was, therefore, sufficient evidence to show that under field conditions desiccation not uncommonly prevents the establishment of myiasis after eggs have been laid.

III. EFFECT OF HUMIDITY ON MATURE EGGS AND YOUNG LARVAE OF *LUCILIA SERICATA* Mg.

Wardle(9) and Evans(2) have studied the effect of humidity on eggs of *Lucilia sericata* over a range of temperatures, but no data appear to be available as to the ability of the larvae to withstand dry conditions. Accordingly, an investigation has been made of the survival of larvae at different humidities. The experiments were conducted at a temperature of 37° C., the mean value ascertained from a series of readings taken at the base of the wool. The relative humidity was controlled by means of a range of dilutions of sulphuric acid (Wilson(10)). The eggs or larvae were placed in a small tube covered above by muslin and suspended in a larger tube which served as an experimental chamber and contained sulphuric acid of the required strength. These chambers were kept in an incubator, the temperature of which was maintained constant by thermostatic control. Table I shows the survival of larvae kept without food at different humidities.

Table I.

*Effect of humidity upon the eggs and larvae of
Lucilia sericata at 37° C.*

Stage	Weight in mg.	Percentage relative humidity			
		50	70	90	100
Eggs: just prior to hatching	...	None hatched	Some hatched	100 % hatched	100 % hatched
Larvae:		Survival in hours			
Newly hatched	0.05-0.01	0.8-1.2	1.5-2.5	4-5	15-21
Late first instar	0.5	1	2	4-5	21-23
Early second instar	0.8	2-25	3-7	7-8	22-24
Mid second instar	2.2	3-45	—	—	—
Late second instar	5.4	11.5-12	—	—	—
Early third instar	9.0	11-12	—	49-56	74
Mid third instar	25.0	25.0	—	—	—

The results in Table I demonstrate clearly the marked effect of dry conditions on eggs (just prior to hatching) and on young larvae. Eggs, which had been incubated under moist conditions and normally would have hatched in $\frac{3}{4}$ hour, completely failed to do so when placed in an atmosphere of 50 per cent. relative humidity, and even in 70 per cent. hatching was not complete. A humidity higher than 70 per cent., preferably about 90 per cent., is essential if eggs are to hatch when in

contact with the skin of the sheep. It is evident also that humidities below 70 per cent. seriously affect the survival of unfed larvae. For instance, the minimum period in which larvae will attain the end of the first instar when reared on meat at the temperature of the sheep's body (39° C.; 102° F.) is 12 hours (Davies⁽¹⁾). The present experiments indicate that, if during those 12 hours the larvae are removed from the food and subjected to humidities of less than 70 per cent., they will succumb in approximately 2 hours. When the larvae had entered the second instar, there was a distinct rise in resistance, which rapidly increased with the size of the larvae. Thus shortly after the first ecdysis they died in 50 per cent. relative humidity within 2.5 hours and in 70 per cent. within 3.7 hours; late second-instar larvae survived in 50 per cent. relative humidity for 12 hours. Third-instar larval stages can withstand still longer exposures to dry conditions.

The survival of larvae at 50 and 100 per cent. relative humidity may be taken to represent their resistance respectively to desiccation and starvation; for death always occurred more quickly at lower humidities and there was no evidence of an optimum humidity below 100 per cent. Table I shows that, as the larvae grow, their resistance to starvation increases progressively, a rapid rise occurring between the early second and early third instar stages; this suggests that the storage of reserve food material begins at this period. Their resistance to dry conditions appears to remain constant during the first instar, abruptly rising as soon as ecdysis has occurred and rapidly increasing during the second instar. The sudden change following ecdysis suggests that water is not lost so rapidly through the new cuticle and spiracles. The increase in resistance to desiccation during the second instar is probably due to the building up of food reserves. Investigations are in progress to determine whether the storage of fat begins at this stage.

IV. STUDIES ON THE HUMIDITY CONDITIONS OF THE WOOL.

(i) *Technique.*

Preliminary attempts were made to ascertain the degree of humidity at the base of the wool by the insertion, close to the skin, of various types of hygrometers, but no satisfactory instrument was forthcoming. An alternative method of determining humidity is to expose and weigh a hygroscopic substance which has been previously calibrated at different humidities, hair being commonly used for this purpose. A modification of this method was adopted. The wool itself is a hygroscopic substance which is suitable for measuring humidity; accordingly, samples were

taken from the base of the wool and transferred to airtight tubes; these were later weighed and exposed to known humidities. From the changes in weight it was then possible to calculate the humidity of the air with which the wool sample had originally been in contact. Gravimetric methods of this type are based on the assumption that the hygroscopic substance absorbs a definite amount of water at a particular humidity. This is not strictly true, as absorption is affected by the past history of the substance. This can be seen in Fig. 1, which shows the changes in weight of wool exposed to increasing and decreasing humidities. The

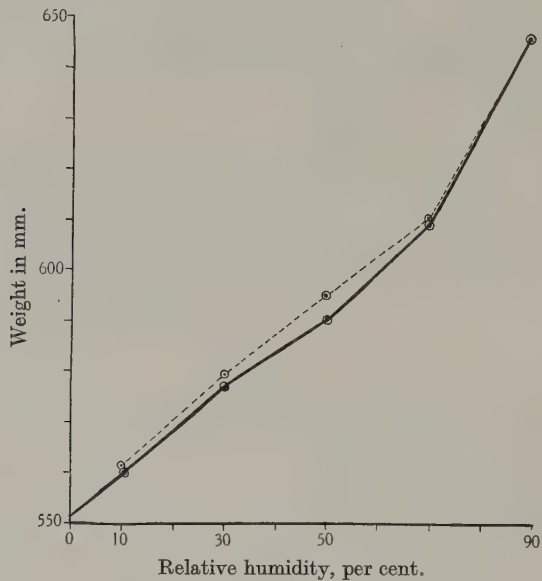


Fig. 1. Curve of weight of sample of sheep's wool at different relative humidities. Full line, rising humidities; broken line, falling humidities.

error, however, is not large, and to eliminate this effect it is usual to approach equilibrium only from one side. Since it was impossible to control the past treatment of the wool samples in this way, they were always exposed first to air of 50 per cent. relative humidity so as to obtain comparative accuracy.

Samples of about 1 gm. were taken from the bottom half-inch of the fleece, care being taken that no wool from the outside was included. Sampling was a comparatively easy matter with long-woolled sheep, but it was difficult to obtain representative samples from sheep having close, crimped wool of the Southdown type. The sample was immediately

transferred to a weighed tube which was closed with a rubber stopper. The tubes were later unstoppered, weighed and then exposed to an atmosphere of 50 per cent. relative humidity. Large museum jars covered with plate glass and sealed with plasticine were used as desiccators. The humidity was controlled by means of sulphuric acid solutions prepared according to the data of Wilson (10). The desiccators were kept in an incubator at 37° C., the average temperature at the site of sampling, since the amount of water absorbed at constant relative humidity varies slightly with temperature; also equilibrium is attained more quickly at 37° C. Under these conditions the tubes reached a constant weight in 3 days, but to ensure that the samples had attained equilibrium they were exposed for at least 4 days. Tubes which *lost* weight at 50 per cent. relative humidity were submitted progressively to air of 65, 80 and 95 per cent. relative humidity until the original weight was reached. Those which did not reach the original weight at 95 per cent. relative humidity were classified as 100 per cent. relative humidity. Tubes which *gained* weight at 50 per cent. relative humidity were exposed to air of 35, and if necessary 20 per cent. relative humidity. In this way two humidities were found, differing by 15 units, between which the original humidity of the sample lay. The exact value was then calculated on the assumption that the relationship between weight and humidity is linear. Fig. 1 shows that this is approximately true over a short range. To test the accuracy of this method, wool samples were exposed to known humidities; they were then treated in the same way as samples brought in from the field. Two samples kept at 25 per cent. relative humidity both gave the theoretical value, 25 per cent.; two kept at 85 per cent. relative humidity gave figures of 84 and 86 per cent. respectively.

(ii) *Range of humidity in the wool at different parts of the body.*

In order to ascertain the general range of humidities at the base of the wool at different parts of the body, ten ewes were selected at random from the flock of sixty pedigree Welsh ewes at the College Farm. Samples of wool were obtained, as described in the technique, from the following parts of the body: between the shoulder blades, the middle of the back, loin, left side, right side, right of the rump and left of the rump.

The results given in Table II fall into three natural groups. Firstly, the humidity in the sample taken between the shoulder blades varied a good deal between the different sheep and ranged from 40 to 80 per cent. It is of interest to note that three out of the ten samples gave values

Table II.

Humidities in the wool of ten pedigree Welsh ewes.

Date: May 9th, 1934. Dull, heavy rain previous night.

Temperature: dry 62° F.; wet 56° F.

Atmospheric humidity: 67 per cent.

Position	No.	Percentage relative humidity										Average
		1	2	3	4	5	6	7	8	9	10	
Shoulder blades	80	60	65	70	45	50	80	45	50	45	59	
Back	45	50	55	50	60	45	45	45	50	40	49	
Loin	55	60	55	65	55	50	60	45	60	45	56	
Left side	50	50	60	65	65	55	60	55	60	55	57	
Right side	70*	70	70	95*	75	65	75*	65	70	70	72	
Right rump	65*	85*	80*	80	85	85*	80*	85*	100*	70*	82	
Left rump	70*	75*	75*	100*	70*	100*	70*	75*	65*	65	76	

* Sample badly soiled by dung.

above 70 per cent., which was higher than the humidity of the atmosphere, whereas in most of the samples taken from other parts of the back the humidity was less than 50 per cent. This indicates that between the shoulder blades some factor exists—possibly excessive perspiration—which may result in moist conditions. It was, in fact, precisely at this point that batches of eggs were found on several sheep. "Shoulder strike" also develops in this region. The second group includes the back, loin and left side, where in all cases low humidity conditions prevailed ranging from 45 to 65 per cent. The third group, namely, the region of the rump, showed high humidities ranging from 65 to 100 per cent., the majority being over 75 per cent. In most cases the wool was soiled with dung, when high humidities were recorded. It was curious to find that the humidities of the wool taken from the right side were higher than the samples from the left side; it is possible that the samples from the right side, which were taken with the right hand and obtained by leaning over the animal, tended to be nearer the rump than those of the left side. This is supported by the fact that three out of the ten samples were soiled with dung.

(iii) *Humidity in the wool under varying weather conditions.*

In order to determine the effect of meteorological conditions on the humidity in the wool, a series of observations were made on four ewes which had given extreme values in the first experiment (ewes 4 and 7, high; 8 and 10, low). Samples were taken from the shoulder, back and loin, the results being given in Table III.

These results show that the relative humidity near the skin of the wool is generally lower than that of the atmosphere. Even following

Table III.

- (a) *Cool, dry conditions. Date: May 23rd. Temperatures: dry 54° F.; wet 48.5° F. Atmospheric humidity: 66 per cent. Wool temperature: 90° F.*

Position	No.	Percentage relative humidity			
		4	7	8	10
Shoulder	43	43	41	43	40
Back	48	46	46	45	39
Loin	47	48	48	46	42

- (b) *Hot, dry conditions. Date: May 31st. Temperatures: dry 73° F.; wet 62° F. Atmospheric humidity: 51 per cent. Wool temperature: 99–105° F. Mean 100.*

Position	No.	Percentage relative humidity			
		4	7	8	10
Shoulder	48	48	39	32	37
Back	55	55	47	44	38
Loin	49	49	44	43	41

- (c) *Showery conditions. Date: August 25th. Ewes had been shorn in mid-July and wool had grown to about 1 in. depth. Temperatures: dry 60.3° F.; wet 54.5° F. (in the screen). Atmospheric humidity: 63 per cent. Samples were taken shortly after showers.*

Position	No.	Percentage relative humidity		
		4	7	10
Shoulder	63	63	47	51
Back	53	53	48	70
Loin	59	59	58	51

- (d) *Wet conditions. Date: August 2nd. Lambs unshorn, and run indoors during showers. Temperature: dry 63° F. (in the screen).*

Position	No.	Percentage relative humidity								
		1	2	3	4	5	6	7	8	10
Back	62	62	72	90	78	65	67	72	73	67

- (e) *Rain at night: light dew when sampling at 9 a.m. Date: August 31st. Lambs unshorn. Temperatures: dry 56.5° F.; wet 53.3° F. (in the screen). Atmospheric humidity: 80 per cent.*

Position	No.	Percentage relative humidity		
		1	2	3
Back	68, 64	68, 64	64, 75	66, 73, 64

heavy rain the humidity remains surprisingly low at the base of the wool; thus, in eight out of ten samples (d) collected from lambs during wet weather after a heavy shower the humidity lay between 62 and 75 per cent. When the outside wool is wet it is difficult to avoid moistening the basal wool when sampling; the lower values are therefore the more reliable.

(iv) *Humidity in wool of different types within the same breed.*

The average flock of mountain Welsh sheep always includes individuals with different types of wool. Table IV indicates that, at least in dry weather, the character of the wool has no effect on the humidity

close to the skin in Welsh sheep. The writers are indebted to Prof. White for the selection of sheep having different types of wool.

Table IV.

Humidity in wool of different types.

Dry conditions. Date: June 12th. Temperature: 45° F. Atmospheric humidity: 68 per cent. Wool temperature: 98° F.

Type	Percentage relative humidity		
	Shoulder	Back	Loin
Kempy	39	42	43
Good Mountain. No kemp	47	44	47
Close Southdown type	42	42	47
Long Slack	40	39	40
"	42	41	39
"	44	45	50

(v) Rate of drying of wool following washing.

Since no data were available as to the rate at which the wool dries after washing, observations were made on three sheep of different wool type which had been immersed for a period of 1 min. in clean water. The results are given in Table V.

Table V.

Rate of drying of wool following washing.

Date: August 22nd. Temperatures: at 9 a.m. in the screen: dry 61.4° F.; wet 56.2° F. Atmospheric humidity: 70 per cent. Intervals of bright sunshine, fair breeze.

Type	Percentage relative humidity (to nearest 5 %)					
	Unwashed control 11 a.m.	Period after washing				
		1 hour	4 hours		8 hours	22 hours
			At base	Outside		
Long wool	50	100	100	75	100	85
Short wool	50	100	100	75	100	90
Medium length	50	100	100	80	100	95

In good drying weather free water was found to be present at the base of the wool for an appreciable period after washing, and even after 22 hours the humidity was over 80 per cent. The observations could not be continued further, since heavy showers interrupted the experiment. There was, however, sufficient evidence to indicate that when sheep are immersed in water the humidity at the base of the wool is favourable to the development of eggs and young larvae of the sheep blowfly for at least 24 hours.

(vi) *Humidity in relation to control.*

In a fleece of average length, such as that of an unshorn Welsh lamb, once the wool base is saturated, as during washing, a considerable time elapses before it dries. Similarly, if the basal wool is wet through urine, faeces or excessive exudation, drying is probably a slow process, and until it has been achieved there is the potential danger of myiasis being established. There is much evidence to justify investigations on control measures which aim either at producing dry conditions at the base of the wool or hastening the drying of a wet fleece, thus attacking the pest at the most vulnerable point in its life history. The fact that ewes are less liable to attack after shearing suggests that the drier conditions of the wool thus obtained are unfavourable to the establishment of myiasis. It was, therefore, decided to ascertain the effect of shearing of lambs on the incidence of attack. A batch of seventy lambs was divided at random into two lots of thirty-five each: one lot was sheared over the entire body, the remainder being left unshorn. The lambs grazed at the College Farm from August 8th to September 4th. Unfortunately the weather conditions during the experiment were unfavourable to heavy infestations and only four lambs were attacked. These four lambs, however, were unshorn. It was worthy of note that four of the shorn lambs, which had scoured badly and would have been regarded as particularly liable to attack, remained unstruck. While the numbers involved were too small to be significant, the results suggest that the shearing of lambs, in the summer, would reduce the incidence of strike. Subsequent to these trials it became known to the writers that in parts of mid-Wales, in the Romney Marsh and doubtless in other parts of the country, it is the practice of some farmers to shear the lambs along with the ewes in early summer solely to reduce blowfly attack.

(vii) *General considerations.*

Since many parasitic insects live close to the skin of man and animals, the humidity near the skin becomes a matter of importance. In the present work observations were made on wool samples taken from the base of the fleece. It has been assumed that the basal wool is in equilibrium with the air near the skin; this is probably true except during violent fluctuations. Several investigators have studied the humidity of the air near the skin of man; the most reliable observations are those of Mellanby⁽⁶⁾, who alone used a suitable method, gasometric analysis of air taken between the shirt and skin. It is of interest, therefore, to note that

the figures obtained with sheep are in good agreement with Mellanby's results on man, and that a similar relationship was found between the humidity near the skin and the external conditions. Table VI shows the values obtained with wool from the back under contrasting weather conditions, humidity being expressed in various ways; some of Mellanby's figures are included for comparison. On August 2nd, when samples were taken after a shower while the outside wool was still wet, the external air was regarded as being saturated. The higher temperature near the skin explains why the *relative humidity* is lower at the base of the wool than outside; for heating increases the amount of water vapour which the air can hold and thereby decreases the ratio of water present to water-holding capacity (relative humidity). When the results are expressed on an *absolute* scale, it will be seen that the air near the skin contains more water vapour than the atmosphere, the excess being due to evaporation from the skin. The results are also expressed in terms of *saturation deficiency*, which shows the drying properties of the air and is calculated by subtracting the absolute humidity from the saturation vapour pressure at the particular temperature. Mellanby, working over a wide range of external conditions, found that this value tended to remain constant under the shirt of man, the extreme values being 13 and 18 mm. The saturation deficiency at the base of sheep's wool appears to be higher and more variable.

Table VI.

	Observations on sheep				Data of Mellanby		
	Date	... May 23rd	May 31st	Aug. 2nd	1	3	
Atmospheric conditions:							
Temperature (° C.)		12	23	17	18	28.4	External conditions
Relative humidity (%)		66	51	100	61	90	
Absolute humidity (mm.)		6.9	12.6	14.4	9.5	2.7	
Saturation deficiency (mm.)		3.6	8.3	0	6	3	
Conditions at the base of the wool:							
Temperature (° C.)		32	38	36	29.6	35	Conditions under the shirt of man
Relative humidity (%)		44	43	68	42	70	
Absolute humidity (mm.)		16	21	30	13	29	
Saturation deficiency (mm.)		20	28	15	17	13	

V. DISCUSSION.

The present experiments have shown that the eggs and young larvae of *Lucilia sericata* are ill adapted to withstand dry conditions at 37° C. This is not surprising, since this blowfly normally breeds in carrion during the early stages of decomposition. If the larvae wander away from the food for any reason, their chances of finding a fresh supply are remote,

especially as most carrion quickly becomes overcrowded. Hence ability to withstand starvation and desiccation would not normally increase the larva's chances of survival in nature. *Lucilia sericata*, besides breeding in carrion, is also a facultative parasite of sheep; fortunately it is not well adapted to this mode of life, the eggs and young larvae being highly susceptible to desiccation. The humidity at the base of the wool of sheep, even in wet weather, is normally so low that eggs may fail to hatch, if laid near the skin, and young larvae rapidly die. Not only can this be inferred from the humidity measurements, but it has also been confirmed by field observations and by direct experiments which will be described in detail in a later publication. These have shown that myiasis is not established unless the surrounding wool is kept wet—first-instar larvae do not produce a wound (which will supply food and moisture) in less than 5 hours. Unfed larvae cannot survive this period at the temperature of the sheep's skin unless the humidity is over 90 per cent. At 31° C., the average temperature at the site of oviposition in wool, eggs hatch in 9 hours (Davies(1)). Therefore, for a successful myiasis on *healthy* skin the humidity must be over 90 per cent. for at least 14 hours (9 hours' incubation of eggs; 5 hours' larval activity); even if a lesion already exists, a high humidity is still necessary since the eggs are extremely susceptible to dry conditions. It is easy to understand why strike so frequently occurs on the rump since the wool in this region is frequently wet owing to soiling with urine and faeces. The cause of "shoulder strike" is more obscure since along the back the humidity at the base of the wool does not usually exceed 70 per cent. even during rain. Larvae would probably fail to establish themselves at this humidity although eggs might hatch if not laid too near the skin. "Shoulder strike", therefore, requires the presence of abnormal conditions, either excessive exudation leading to a high humidity or a lesion which would supply the larvae with food (and moisture) on slight irritation.

The above considerations have shown that a high humidity is necessary for the development of eggs and young larvae of *Lucilia sericata* on sheep. Bacterial action also plays an essential part by attracting the fly to oviposit. Seddon(8) has shown that "body strike" in Merinos is often caused by "water rot"—an infectious condition of the wool which is due to bacteria. It will be shown in the following paper on the tropisms of blowflies that most sources of ammoniacal decomposition stimulate *Lucilia sericata* to oviposit on sheep. Wool contains large numbers of bacteria and there can be little doubt that the presence of moisture will lead to bacterial decomposition in the fleece. It is probably not over-

stating the case to say that, given sufficient sunshine and a suitable temperature for the normal activities of the blowflies, the humidity at the base of the wool is *the factor* which determines susceptibility.

VI. SUMMARY.

The present paper is intended as the first of a series dealing with various aspects of the sheep maggot problem, especially the causes of susceptibility. Since this pest is more common in moist weather, an investigation was made of the effect of humidity on sheep maggots; also, a method was devised for studying the humidity at the base of the wool—the site of attack.

The following is a brief summary of the results obtained:

(1) The eggs and young larvae of *Lucilia sericata* Mg., the sheep maggot fly, are extremely sensitive to desiccation at 37° C.

(2) Dry conditions prevail in the fleece near the skin, except in the region of the rump, and the relative humidity seldom exceeds 70 per cent., even during wet weather.

(3) These results show that the microclimate at the base of the wool is normally too dry for the development of maggots; this has been confirmed by finding desiccated eggs and larvae on sheep in the field.

(4) Sheep do not become infested with maggots unless predisposing conditions are present; the humidity at the base of the wool appears to be the main factor which determines susceptibility.

(5) The wool around the rump is often moist owing to soiling with faeces and urine, the humid conditions favouring the development of eggs and maggots.

This work has been rendered possible by a grant to one of us (R. P. H.) from the Agricultural Research Council, to whom we tender our best thanks. We wish, also, to express our appreciation to Prof. R. G. White for his interest and helpful suggestions in this work and for the facilities provided at the College Farm.

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SHEEP BLOWFLY INVESTIGATIONS

II. SUBSTANCES WHICH INDUCE *LUCILIA SERICATA* MG.
TO OVIPOSIT ON SHEEP

BY R. P. HOBSON, B.Sc., PH.D.

*(Department of Agricultural Zoology, School of Agriculture,
University College of North Wales, Bangor.)*

INTRODUCTION.

THE infestation of sheep with larvae of *Lucilia sericata* Mg. (the sheep maggot fly) causes severe losses to farmers in Great Britain and other countries. This blowfly normally breeds in carrion, but it also oviposits in the wool of live sheep, and the resulting myiasis produces serious wounds which may prove fatal. Seddon (10), working in Australia, showed that a sheep is not struck unless it is in a susceptible condition; also, he determined the main causes of susceptibility in Merinos. For a successful strike to occur, it is evident that (a) the fly must oviposit on the sheep, (b) the larvae must be able to develop. The present paper describes experiments on substances which attract flies to lay their eggs on sheep. It has long been suspected that sheep have a specific attraction for *Lucilia sericata*, and various hypotheses have been advanced to account for the origin and cause of this habit.

OBSERVATIONS AND EXPERIMENTS.

When a sheep is badly infested with *Lucilia* larvae, the wool in the affected region becomes stained with a dark, evil-smelling fluid, which is mainly composed of the excretions of the larvae. If the weather is warm and sunny, the stained wool attracts sheep maggot flies, and further eggs are laid in large numbers. Experiments with healthy sheep have shown that they can be rendered attractive to *Lucilia* by placing larval excreta in the wool; other substances also proved effective. Although *Lucilia* oviposits on treated sheep, the larvae fail to grow as the environment is unsuitable; for myiasis to occur, other predisposing conditions must be present.

Methods used.

Most of the experiments were carried out with Welsh sheep in a small paddock behind the laboratory. About 5 c.c. of the solution to be tested were poured on to cotton-wool placed in the wool on the back; after

2 hours the sheep was examined for the presence of eggs. Positive controls were always carried out with a substance known to be active. Experiments were only made in bright, sunny weather; if the temperature in the sun was over 67° F. (19.4° C.) the results were reliable. Oviposition was not found to occur at temperatures below 65° F. (18.3° C.).

Experiments with larval excreta.

Faeces of *Lucilia* larvae can be obtained by allowing water to drip slowly through a filter containing half-grown larvae; the product is a dark brown liquid which contains free ammonia and trypsin (4). A large amount of this substance was prepared and stored in a stoppered bottle, the solution remaining attractive for several weeks. Although the larval excreta attracted blowflies, this substance *alone* did not induce egg-laying either in the field or in a cage containing large numbers of gravid females. However, when placed *on live sheep*, the excreta invariably induced oviposition on warm, sunny days. Generally six or more females would lay within 2 hours, according to the size of the treated area. As many as 3000 eggs have been obtained in one day when several sheep were used; this was calculated from the number of egg batches on the assumption that each female lays 100 eggs. The regularity with which the flies responded was striking evidence of the density of the fly population in the field. This was not due to a local high concentration of flies, as similar results were obtained at the College Farm, which is 6 miles distant.

This chemotropic response raises two points of considerable biological interest: (1) only females of the species *Lucilia sericata* respond, whereas carrion attracts other blowflies besides *Lucilia*; (2) the living sheep plays an essential part. There was no evidence in these experiments that flies other than *L. sericata* oviposited on the sheep. Many of the eggs collected were bred out on meat, and the emerging adults examined; also, observations in the field showed that other common blowflies, such as *Calliphora* and *Sarcophaga*, were not attracted to sheep treated with the substances used in these experiments. The significance of this will be discussed later.

Table I summarises the results of the tests with larval excreta. These show that the excreta were only effective in close proximity to live sheep, direct contact not being necessary. Neither the wool nor skin could take the place of the live sheep, and oviposition did not occur with other animals. When the hide of a recently slaughtered sheep was placed in

the field, no eggs were laid on the spots treated with larval excreta; however, oviposition occurred on the following day on the skin derived from the hind-quarters, which were soiled with dung and urine. Two experiments were carried out with larval excreta placed in a tube which contained cotton-wool and was fastened upright on the back of a sheep; eggs were laid around the tube on one occasion and inside it on the other. The effect of the excreta is not, therefore, due to chemical or bacterial changes set up on the skin surface or in the wool. The attraction seems to consist of a combination of two sets of factors, one supplied by the sheep and the other by the excreta.

Table I.

Chemotropic experiments with larval excreta.

Site where excreta were placed	Oviposition
Wool of live sheep	+
Skin of recently shorn sheep	+
Tube fixed in wool of sheep	+
Cotton-wool	-
Sheep's wool	-
Skin and fleece from dead sheep	-
Human arm (in cage)	-
Skin of live cow	--

These results show that *Lucilia sericata* can recognise *live* sheep in some way; also, the fly is able to distinguish a live sheep from the wool and hide of a dead animal. Since blowflies are very responsive to odours, the natural smell of the sheep is probably the chief factor. Some evidence for this was provided by the observation that eggs were laid more readily on rams, which smelled more strongly to the human perception. Thus, larval excreta diluted 100 times induced oviposition on rams, but not on lambs. It is of interest to note that flies laid freely on a Wiltshire lamb in the presence of larval excreta, although this breed is practically immune from sheep myiasis.

Experiments with other substances.

It was found that other substances produced the same effect as larval excreta; although they were ineffective alone, *Lucilia* would oviposit on them when incorporated in the wool of live sheep. Table II shows the results of these experiments.

Pure water and sterile bacteriological broth had no effect within 2 hours, but broth cultures inoculated from larval faeces and incubated for 4 days at 37° C. readily produced oviposition on sheep. However, the attraction of these cultures for *Lucilia* flies was not due to the use of bacteria derived from *Lucilia* larvae, since mixed cultures of organisms

obtained from the air and from sheep dung proved equally effective. Fresh sheep dung and urine gave negative results, but they became attractive after incubation. Faeces from sheep suffering from diarrhoea were effective without previous incubation; it should be noted that scouring is a very frequent cause of sheep strike in this country. It was probably significant that faeces from scouring sheep were strongly alkaline, whereas normal dung and urine were approximately neutral when fresh. Ammonia proved slightly attractive in high concentration, but the introduction of methyl and ethyl groups did not increase the activity; indole was ineffective. The attractiveness of larval excreta was destroyed by making the reaction faintly acid (*circ.* pH 5.0) and by chemical fractionation, but not by sterilising in an autoclave. After steam distillation with magnesia, both the residue left in the flask and the distillate were inactive; after concentration in acid solution, the distillate had no effect when tested at an alkaline reaction.

Table II.

Ability of different substances to induce oviposition on sheep.

Excreta of <i>Lucilia</i> larvae	+++	Sheep dung	-
Beef extract broth (sterile)	-	Incubated dung	+
Broth infected from larval excreta	++	Dung from scouring sheep	++
Broth infected by exposure to air	++	5 % ammonia	+
Broth infected with sheep dung	++	1 % ammonia	-
Broth infected with organism derived from <i>Lucilia</i> larvae	++	1 % tri-methylamine	-
Fresh urine (sheep)	-	1 % ethylamine	-
Incubated urine	+	Indole	-

These results suggest that any source of ammoniacal decomposition¹ will induce *Lucilia* to oviposit on sheep. The specific attractiveness for *L. sericata* is, therefore, not due to the nature of the putrefactive products, but to the special factors, whatever they may be, which are supplied by sheep and which *L. sericata* is able to recognise. The chemotropic effect of larval excreta and other putrefying substances is no doubt caused by a variety of compounds which together produce an odour and taste which stimulate *Lucilia* to oviposit on sheep. The fact that an alkaline reaction is essential suggests that basic compounds play an important part.

¹ In a recent account of the sheep maggot problem in Scotland (*Scot. J. Agric.* xvii, 249-60), Prof. J. Ritchie records that many farmers, in replying to a questionnaire, blamed starlings for encouraging this pest; also one or two produced definite evidence, having actually found batches of eggs in the fleece close to starlings' droppings. The present experiments suggest that this material is likely to induce oviposition on sheep, but the season was too advanced for field tests when Prof. Ritchie's paper was seen.

DISCUSSION.

The present experiments show that *Lucilia sericata* readily oviposits on sheep in the presence of various substances which by themselves do not stimulate the fly to lay. These include excreta of *Lucilia* larvae, faeces from scouring sheep and broth cultures infected from various sources. An alkaline reaction seems to be essential, since larval excreta became unattractive when made faintly acid. Ammonia alone proved slightly effective; it seems probable, therefore, that any source of ammoniacal putrefaction will stimulate *Lucilia* to oviposit on sheep. These results explain the initial attraction which leads to fly attack; also, the rapidity with which infestation increases on an affected animal, since larval excreta proved the most effective substance tested.

The attraction of sheep for *L. sericata* seems to differ from that provided by carrion. In Great Britain(2, 9) sheep myiasis is almost exclusively caused by *L. sericata*, and this was the only species observed to oviposit on sheep rendered artificially attractive. Various blowflies and other insects breed in carrion. Davies(2) noted that *Alysia manducator* is not attracted to *Lucilia* larvae developing on live sheep, and confirmed the views of Altson(1) that this parasite is primarily attracted by decomposing carrion. However, in Australia(8) several species of blowflies infest sheep. Six species (primary flies) can initiate sheep strike, *L. cuprina* being the chief offender and *L. sericata* second in importance; also other species (secondary and tertiary flies) follow up the attacks of the primary blowflies. It would be of interest to know how the different blowflies in Australia respond to the substances used in these experiments.

Various theories have been advanced to explain the origin of blowfly strike in sheep. Froggatt(3) suggested that strike is due to a change of habit in the blowflies themselves. Johnston(7) and Holdaway(6) advanced the idea that strike is not an acquired habit, but the natural response of blowflies to bacterial activity in the wool. The present experiments have shown that healthy sheep provide an essential stimulus which, in conjunction with bacterial activity, attracts *L. sericata* to oviposit. They support Froggatt's theory that sheep strike is due to an acquired habit on the part of the fly, but they also confirm the views of Seddon(10) and Holdaway(6) that bacterial action is a primary cause of fly attack.

The incidence of strike is so sporadic that up to now it has been difficult to determine the effect of preventive measures, and field trials must be continued for several weeks with large numbers of sheep. Ex-

perimental methods of producing strike are therefore desirable. These experiments have shown how sheep can be made attractive to *L. sericata*, and this discovery should provide a convenient technique for testing repellents under field conditions. A considerable amount of work has been done in Australia on trapping blowflies; whether or not trapping is a practical proposition in Great Britain, this method of attracting flies represents the ideal way from the scientific point of view since only *L. sericata* respond. Carrion baits are not specific and attract other blowflies which compete with *L. sericata* in nature. The amount of available food is probably the main factor which determines the total blowfly population; thus, according to Holdaway⁽⁵⁾, only a small proportion of the *L. sericata* larvae present on carrion reach maturity, owing to overcrowding and competition from other species. The present form of attraction would act differentially against this one species harmful to sheep, if it could be utilised for trapping purposes, and the possibility of this is being investigated.

SUMMARY.

1. Various putrefying substances attract *Lucilia sericata* to oviposit on sheep *in the field*. Other blowflies do not respond.
2. Excreta of *Lucilia* larvae are extremely attractive; faeces from scouring sheep, stale urine and bacterial cultures are also effective.
3. *Lucilia* does not oviposit on these substances unless they are placed close to the skin of a live sheep; neither sheep skin, wool, nor other live animals can take the place of sheep.
4. The attraction is not due to chemical or bacterial changes in the fleece, since actual contact with the skin or wool is not necessary.
5. It is therefore concluded that the sheep itself plays an essential part in attracting *Lucilia*. The natural odour may be responsible.
6. These results are discussed in relation to the origin and control of sheep strike.

I am greatly indebted to the Agricultural Research Council for a grant which has entirely financed this work. I wish to express my appreciation to Prof. R. G. White for extending to me the facilities of the School of Agriculture, also to Dr W. M. Davies for much helpful advice and for identifying the specimens of blowflies.

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STUDIES ON THE SECRETION OF DIASTASE AND INVERTASE BY *EMPOASCA SOLANA* DELONG (RHYNCHOTA, HOMOPTERA, JASSIDAE)

By G. V. B. HERFORD, M.Sc.

(*Biological Field Station, Imperial College of Science and Technology.*)

THE work to be described was carried out at the Graduate School of Tropical Agriculture at the University of Hawaii, and the writer wishes to express his indebtedness to Prof. R. N. Chapman and Dr Walter Carter for their constant interest and for much valuable advice.

Since the time available was limited, it was not possible to carry out a thorough investigation of the problem, but it is hoped that the experiments and technique may be of interest to others working in the same field.

The leaf-hopper, *Empoasca solana* DeLong⁽³⁾, causes leaf-burn in a considerable number of cultivated plants grown in Hawaii. It was chosen for experiment on account of its great abundance on castor-oil plants which grow as weeds in the grounds of the university. Adults and nymphs could be collected in unlimited numbers from the undersides of the leaves by means of a sucking tube. Before handling, the insects were stupefied with a very light dose of ether, which did not appear to exert any harmful effect on them.

I. DIASTASE.

Davidson⁽²⁾, Horsfall⁽⁴⁾ and others, experimenting with aphids, by dissecting out the salivary glands and placing them upon starch agar, have shown that when the agar is later treated with iodine those spots where the salivary glands had rested remain unstained, thereby indicating the presence of diastase in the salivary glands.

Is diastase actually ejected into the food?

Glass tubes, $1\frac{1}{4}$ in. in diameter, were prepared with 10–15 ml. of starch agar and autoclaved for 20 min. at 15 lb. pressure with sufficient paraffin wax to form a thin film on the surface. One tube was kept as a control and the others were provided with ten leaf-hoppers per tube. The tubes were plugged with cotton-wool wrapped in muslin and laid horizontally, with the agar towards the light.

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The insects congregated on the paraffin membrane and could be seen to be feeding through it. After 24 hours the insects were removed, the tubes were treated with ether to dissolve the wax and the agar in each was boiled for 5 min. with 15 ml. of Fehling's solution to test for the presence of reducing sugars.

Result.

Control tube	...	No precipitate, or any discoloration.
Feeding tubes	...	Heavy reddish yellow precipitate in each tube, indicating the presence of reducing sugars.

These results, which were confirmed in repeat experiments using 3 per cent. starch solution, show that diastase can be ejected by these insects when feeding.

Is diastase secreted only by the salivary glands?

Carter and Cotner⁽¹⁾ have demonstrated the presence of yeasts in sugar solutions on which leaf-hoppers (*Eutettix tenellus* Baker) had been feeding through a membrane, and have shown that these yeasts, presumably injected by the insects, had the power of rotating certain sugars. It appeared possible, therefore, that yeasts might be regurgitated from the fore-gut of the insects together with the saliva, and might themselves be a source of diastase.

Accordingly, several tubes of starch agar were prepared with paraffin films, autoclaved as before, and provided with 15-20 leaf-hoppers per tube. After 4 days there was a distinct growth of yeasts below the paraffin membrane.

Smears and stabs were made of the yeasts on to freshly poured plates of sterile starch agar. Of five plates so inoculated, three showed traces of outside contamination after 3 days; these were discarded. One control plate, uninoculated, showed no contamination. The remaining two plates showed only the yellow yeast colonies. One of these plates was treated with iodine solution, the yeast colonies having been previously scraped off. Only those spots where the yeast colonies had been growing remained unstained, the remainder of the agar showing the typical deep blue coloration.

Circles of agar were cut with a test tube from the stained and unstained portions of the iodine-treated plate and boiled for 5 min. with 10 ml. of Fehling's solution. Only the very slightest precipitate came down, even in the case of the unstained agar where the yeast colonies had been. This indicated, possibly, that the starch had been as yet only partially hydrolysed by the enzyme.

The remaining plate was therefore kept for a further 2 days in the hope of obtaining complete hydrolysis. At the end of this time 1 sq. in. was cut from the agar below the yeast growths and a similar area was cut from a portion of the plate well removed from the colonies and the two samples were each boiled for 5 min. with 10 ml. of Fehling's solution. A third sample was cut from the control plate and similarly treated.

Result.

Agar under yeasts	Definite red precipitate.
Agar away from yeasts	Very slight red precipitate.
Agar from control plate	No precipitate.

This shows that: (1) the yeasts secrete diastase, and (2) that in the plate 4 days old the effect of the diastase had diffused through the agar to a certain extent. It should be mentioned, however, that treatment with iodine did not reveal any noticeable gradation between the stained and unstained areas, the line of demarcation being quite sharp. This may possibly be due to the fact that diffusion occurred in the body of the agar, while the iodine affected only the surface. It remained still to be shown that similar yeasts could be found in the gut of the insects and that these yeasts also secrete diastase.

A number of leaf-hoppers were killed by quick immersion in ether; the alimentary canals of ten of these were successfully dissected out in physiological saline. (The salivary glands were, of course, discarded.)

The alimentary canals were inoculated on to a sterile plate (*A*) of starch agar. Another similar plate (*B*) was inoculated with saline only. After seven days a good growth of yeasts was visible on the inoculated plate (*A*). Plate *B* showed a certain amount of bacterial growth, grey in colour, and entirely different from the yeast cultures, showing that the saline had not been completely sterile when used. A control plate (*C*), stabbed with a sterile needle, showed no contamination.

Equal amounts of agar from plates *A* and *B* were boiled with 15 ml. of Fehling's solution for 5 min.

Result.

Plate <i>A</i> agar	Heavy red precipitate.
Plate <i>B</i> agar	No precipitate or discoloration.

It appears, therefore, that certain yeasts, having the power to secrete diastase, are present in the alimentary canal of these insects, and that some of these yeasts are regurgitated with the saliva into the food substance.

II. INVERTASE.

In all the experiments described, involving the use of sucrose solution, the following procedure was adopted as standard.

In preparing the sucrose solution, the crystals were first washed in absolute alcohol (since autoclaving was found to cause inversion) and dissolved in autoclaved distilled water. A 5 per cent. solution was used throughout. All glass tubes were sterilised with absolute alcohol before the sucrose solution was added.

In testing the solutions for inversion into reducing sugars, Striegler's modification of Fehling's solution was used (5). This solution is claimed to be more sensitive than Fehling's in determining minute quantities of dextrose in the presence of sucrose. The formula is as follows:

K_2CO_3	...	125 gm.
$KHCO_3$...	50.7 gm.
$CuSO_4$...	3.464 gm. in distilled water; dilute to 500 ml.

Equal parts of the sugar solution and of Striegler's solution (usually 15 ml. of each) were taken and boiled for $\frac{1}{2}$ min., the flame being removed at the end of that time and the solution allowed to cool slowly.

No attempt was made to estimate the weights of the copper precipitates which were formed, as it was felt that no useful end would be served in so doing, since it was impossible to tell how many hoppers had been actually feeding, and for how long.

Considerable difficulty was experienced in perfecting a technique for feeding leaf-hoppers upon a solution. Experiments with paraffin membranes on the surface of the liquid showed that any film thin enough to allow the insects to feed through it was so weak as to break with any movement of the liquid, such as must occur in handling the tube.

Accordingly, a modification of the "fish-skin" technique described by Carter and Cotner (1) was used. The skins are extremely thin animal membranes, which are used for medical purposes. Portions of the membrane are cut and fastened by surgical plaster over the top of a glass tube containing the liquid. A similar tube, but open at both ends, is attached by plaster over the membrane, and the open end closed with a plug of cotton-wool wrapped in muslin.

It was found necessary to soak the membranes in carbon tetrachloride before using, to dissolve out the grease with which they had been impregnated, and which hampered the hoppers in feeding. Even so, however, the control tubes, *i.e.* those fitted with a membrane but containing no hoppers, gave a positive reaction with Striegler's solution, showing

that inversion of the sucrose was taking place. Sterilisation with absolute alcohol, to get rid of a possible bacterial contamination, made no improvement.

It was considered, therefore, that there must be some enzyme actually present in the fish-skin membranes themselves which was causing inversion in the control tubes. The following experiment was therefore set up to investigate this point:

15 ml. of sterile sucrose solution was poured into each of four tubes, which were fitted with membranes prepared as follows:

Tube 1. 1 sq. in. of membrane previously boiled for 4 min. in distilled water.

Tube 2. 1 sq. in. of membrane previously soaked for $\frac{1}{2}$ hour in 5 per cent. mercuric chloride, and washed in four changes of distilled water for 45 min.

Tube 3. 1 sq. in. of membrane, untreated except for "degreasing" in carbon tetrachloride.

Tube 4. Control; no membrane added.

(The squares of membrane in tubes 1-3 were placed actually in the sucrose solution, in order to give the maximum effect.)

After 4 days, the solution in each tube was poured off and boiled with Striegler's solution, with the following results:

Tube 1. Slight, but definite red copper precipitate.

Tube 2. No precipitate.

Tube 3. Very heavy red copper precipitate.

Tube 4. No precipitate.

It appeared, therefore, that an enzyme was present in the membranes, and that treatment with mercuric chloride would "kill" it.

Working on these results, the following technique was used. The membrane was cut into 2 in. squares, "degreased" for 2 hours in carbon tetrachloride, soaked for $\frac{1}{2}$ hour in 5 per cent. mercuric chloride, washed for 2 hours in running tap water and $\frac{1}{4}$ hour in fresh distilled water, soaked for 5 min. in 90 per cent. alcohol and 5 min. in absolute alcohol, dried quickly over a flame and fastened over the tube with surgical adhesive tape.

Five tubes were prepared in this way, each containing 20 ml. of sterile sucrose solution. One was kept as a control, and 30-40 hoppers were added to each of the others. The insects were allowed to feed for 3 days, after which the solution in each tube was boiled with an equal amount of Striegler's solution.

Result.

Tube 1 (with insects)	...	Heavy red copper precipitate
Tube 2 ,,	...	,, ,,
Tube 3 ,,	...	,, ,,
Tube 4 ,,	...	,, ,,
Tube 5 (control)	...	No precipitate

It appears, therefore, that these insects are capable of inverting sucrose into reducing sugars by means of an enzyme.

III. SUMMARY.

1. It has been shown that adults and nymphs of the Jassid, *Empoasca solana* DeLong, are able to eject diastase into the feeding medium.

2. This diastase, besides being secreted in the salivary glands, is apparently also formed by certain yeasts which are regurgitated from the gut of the insects.

3. A technique is described for feeding the leaf-hoppers on a liquid.

4. The insects have also been shown to be capable of secreting an enzyme, probably invertase, which has the power of inverting pure sucrose.

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THE COMPARISON OF DOSAGE-MORTALITY DATA

By C. I. BLISS.

(*Galton Laboratory, University College, London.*)

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DOSAGE-MORTALITY data in toxicological experiments may be defined as those in which the action of a given amount of a toxic agent is measured by the proportion of treated organisms in one of two distinct categories, such as dead and alive. For any given toxic agent and type of organism, the relation between dosage and mortality can be established from a series of such tests. Methods have been described (15) by which we may plot the original data as a straight line, estimate with accuracy the most probable position of the dosage-mortality curve, and determine within any given odds the zone on each side of this computed curve within which

our estimate may be in error. In the present paper we shall assume that a similar dosage-mortality curve or its equivalent is available as a standard of reference, and will concern ourselves with the problems which arise (a) in determining whether other records of one or more experiments agree with the standard within the limits of sampling error, and (b) in estimating dosage from mortality and the error of such estimates of dosage. We will consider tests which are appropriate when the standard curve has an absolute accuracy or no zone of error, when it has been determined from data agreeing within the limits of sampling error, and when it has been estimated from more variable data which exceed these limits. Since the statistical methods for making these tests are a further development of those used in the preceding paper (15), to which reference should be made, the numbering of the equations, tables, and citations from the literature continues each its respective series.

MEASURING THE AGREEMENT BETWEEN DOSAGE-MORTALITY DATA.

While the exact measurement of the relation between the dosage of a given poison and the mortality which it produces is often of importance, dosage-mortality experiments are more frequently comparative in type. In the search for new insecticides, for example, the relative effectiveness of many different preparations is more interesting than isolated statements of how individual compounds may act. In other instances the method of preparation of a complex drug may be under investigation, and the question of whether or not slight differences in toxicity are significant may be of considerable importance. The comparative effectiveness of an established poison under different environmental conditions, such as of temperature and humidity, may be investigated, or the relative susceptibility to a particular poison of different species, races, or developmental stages of an insect may demand study. In these and other instances it is often necessary to determine whether closely similar results agree or not within the limits of experimental and sampling error, and if they do not agree, with what precision the difference between them has been established. The extension of the statistical methods which have been developed for analysing a single series of records to the comparison of such data is, therefore, of some importance.

It is essential in making comparisons of this type that in at least one series enough different dosages be used to compute the dosage-mortality curve and its errors. With this as a standard, the susceptibility to other treatments can be compared on a uniform basis, even in cases where only a single dosage has been applied. The results, however, are always

more reliable when the mortality has been measured at two or more dosages, which should be planned, so far as possible, to fall within the range giving from 40 to 90 or 95 per cent. kill. The fact that in many instances compounds have been compared on the basis of a single, or at most two, dosages, or only the concentrations giving the difficultly determinable 100 per cent. kill have been reported, emphasises the need for these experimental prerequisites if the determinations of mortality are to have a reliability at all comparable to the accepted standards for measuring chemically the dosage applied. The statistical tools which are appropriate for any given comparison will depend largely upon the character of the dosage-mortality curve which may be designated as the "standard", and accordingly will be so classified.

Comparisons with a standard curve of absolute accuracy.

The comparison of one or more observations with a standard is simplified if the standard itself is free of possible error. This condition, however, will be infrequent at best. It would occur if the true dosage-mortality curve could be established from theoretical considerations. The mere transformation of dosage units to logarithms and of percentage mortalities to probits, despite the theory underlying this transformation, does not turn the regression line into a theoretical curve, for it is still determined entirely from the same units of which it is a description. In a true theoretical dosage-mortality curve, which would be free of sampling error, either the parameter for position, a , or that for slope, b , or both, must be fixed by observations or theoretical computations not based upon the percentage mortalities measured after the application of known dosages of poison. If a true theoretical curve which met this requirement could be determined, it would have an absolute accuracy such that the larger the number of observations and individuals used in computing an equivalent empirical curve by the methods described in the preceding paper, the more nearly would this second empirical curve coincide with that based on theory. In the present stage of toxicological research, no theoretical curve has yet been established, so that every standard curve has a finite rather than an absolute accuracy.

There are instances, however, such as in the biological assay of important drugs, of insecticides, or of X-ray tubes, where the exact position of the standard curve is of considerable importance and may be established by such a large number of observations under such rigidly controlled experimental conditions that its zone of error becomes exceedingly

small relative to the sampling error in the biological material of a given assay. If computation has demonstrated that these errors are actually negligible, which is seldom the case, the zone of error of the empirical standard may be disregarded and the curve treated for practical purposes as if it had an absolute accuracy. When the standard curve has an unknown zone of error, it must necessarily be treated as if it had an absolute accuracy, although the reliability of the result is not comparable with that in the preceding case, where the zone of error is demonstrably negligible.

The case of a single observation. The statistic most appropriate for comparing an observed percentage mortality with that which would be expected at the same dosage from a standard curve of absolute accuracy is the χ^2 test. If the standard curve is given in probits, the probit corresponding to the dosage in question is first converted to terms of the proportionate mortality, p , by means of Table I, and the observed mortality in our new experiment handled as a single sample from a binomial distribution with p as the true proportion dead. The χ^2 test then takes the form

$$\chi^2 = \frac{(D - pN)^2}{pqN} \quad \dots\dots(13),$$

with one degree of freedom, when, in the sample, D =number of individuals killed, N =number of individuals exposed to death, p =proportion expected to die at that dosage as determined from the standard curve, and $q=1-p$. If χ^2 is larger than 3.84, the odds are less than 1 in 20 that the new observation represents the same toxicological relationship as the standard curve.

An approximate estimate of χ^2 may be secured directly from the probit value of the new observation, y_1 , and that of the theoretical curve, Y , at the same dosage:

$$\chi^2_{\text{approximate}} = (y_1 - Y)^2 w_1 \quad \dots\dots(14),$$

when w_1 is the weighting coefficient (Table III) for the theoretical expectation (Y) multiplied by the number of individuals used in determining y_1 . Its accuracy depends upon the applicability of the methods of large samples. Since the approximate χ^2 is larger than the true value, if it indicates that a given observation is in agreement with a theoretical curve, the conclusion can be accepted as reliable. However, if it indicates a discrepancy with P between 0.01 and 0.05, the computation should be repeated with the exact equation (13).

The case of several observations. When experiments have been made at several dosages in the new series, the χ^2 (or χ) for each observation is

computed separately and the results added to secure a test for the entire series. The exact form of the test, however, will depend upon whether the new observations differ from the standard principally in slope or in position. If the departure from expectation is at one end of the curve positive and at the other end negative, so that it is primarily a difference in slope, the χ^2 test will measure whether the amount of this departure exceeds the variation to be expected by chance. In this case the χ^2 for each observation is computed from equation (13) and then they are added to give a combined χ^2 for the entire series, that is entered in the χ^2 table, such as Table III in Fisher's text, with as many degrees of freedom, n , as there are separate values of χ^2 included in the sum. If the combined χ^2 corresponds to a value of P smaller than 0.05, the new series probably is not consistent with the theoretical standard.

This test, however, depends only on the amount of departure from expectation and not on the direction of this departure. If the new experiment which is being compared with a previously established curve shows on the whole a consistently larger or smaller mortality, this fact is of importance and should enter into the determination of agreement. In such a case we may utilise the fact that the sum of the separate χ 's, $S(\chi)$, is normally distributed with a variance equal to the sum of the degrees of freedom. To test for agreement, χ is determined for each observation as follows:

$$\chi = \frac{D - pN}{\sqrt{pqN}} \quad \dots\dots(15),$$

so that observations above the standard dosage-mortality curve are positive and those below the standard negative. These are then added with respect to sign, and the sum, $S(\chi)$, which may be either a positive or a negative quantity, is divided by the square root of the number of χ 's, \sqrt{n} , to test for significance. If the quotient $\frac{S(\chi)}{\sqrt{n}}$ exceeds 1.96,

the odds are less than 1 in 20 ($P=0.05$) that the new series represents the same relationship as the standard curve. Values of P , the probability integral, for any experimentally determined quotient or "deviate" are given in Tables I and II of Fisher's text.

If the new observation show an agreement with the standard with one test but not with the other, a discrepancy in the χ^2 measure may be attributed to a difference in slope or the relative uniformity in the susceptibility of the population of organisms, a discrepancy in the $S(\chi)$ to a difference in position or the level of susceptibility of the population as a whole. When the new observations differ significantly from the

standard by both measures, a discrepancy in position is established with certainty, but not necessarily one in slope.

Comparisons with a standard curve determined from homogeneous data.

For quantitative toxicological determinations, the most satisfactory comparisons are those with standard curves which have an accuracy dependent entirely upon the unavoidable error of sampling. This condition is fulfilled when the dosage-mortality curve has been transformed to a rectilinear form, a regression line fitted by the methods described in the preceding paper, and agreement within the limits of sampling error between the experimental observations and the fitted line determined by the χ^2 test (equation (7)). Under these conditions, the homogeneity of the data is assured, in so far as this can be ascertained from the number of tests and "weight units" that have been used—"weight units" (as defined by equation (1)) being specified rather than "number of organisms" because of the reduced importance of tests at high and low dosages. With large numbers of tests and weight units, the sampling error is reduced to a point where it cannot mask any other sources of variation, and if the χ^2 test still indicates homogeneity, the experimenter can be sure that for the toxic agent in question his stock of experimental animals and his methods of experimentation cannot be further improved. When determined from homogeneous data, the standard curve will be subject to two sampling errors, an error in position computed from equation (10) and one in slope calculated from equation (11). The problem is to decide whether the mortality observed in a second or parallel experiment is consistent with a standard curve that has been established within an accuracy limited by these errors. In other words, could the mortality in the second record, whether based on tests at one or many dosages with few or many individuals, occur if it were governed by the same toxicological relationship as that given within the zone of error of the standard? This is accomplished if we determine whether or not the discrepancy between the new records and the standard is statistically significant.

Graphic comparisons. Sometimes graphic comparisons may be sufficient without further statistical computations. The new observations, in terms of logarithms and probits, may be spotted on a diagram of the standard regression line which shows the limits of accuracy within which it has been determined, such as the curved lines in Fig. 3 of the preceding paper (15). If the additional determinations fall within these curves bounding the zone of error of the original standard, they may be con-

sidered as equivalent to the original samples from which the curve was computed. Or if the mortalities fall at random above and below those in the standard, as in Series I and II of Fig. 3, a statistical calculation may not be necessary to confirm their equivalence. Nor, of course, is it essential for satisfactory agreement that all observed values fall within the curves which delimit the error in estimating the standard regression line, a condition violated by two out of the twelve observations in the main curve of Fig. 3. But if most points fall outside the zone of error, and if the new series is based upon four or more separate determinations of mortality at three or more different dosages, the data can be used for a new estimate of the "true" dosage-mortality curve and its zone of error. Should the two zones fail to overlap over any part of the range of observed dosages, the two series cannot be considered as identical. Although graphic methods such as these will answer certain of the requirements of the investigator, an arithmetical approach is frequently more rapid and usually to be preferred.

Indirect χ^2 tests for the discrepancy of additional observations. Indirect χ^2 tests for the discrepancy of individual observations can be applied by means of the equations which have already been given. Through the application of equation (7), it has been established, presumably, that the standard curve agrees within the limits of sampling error with the observations from which it has been computed. If, subsequently, one or more further experiments become available, we may pool both the original and the new records into a single series and recalculate the standard curve so as to include these additional tests, and again apply the χ^2 test given in equation (7) to determine similarly whether the second curve is in agreement with the increased number of observations.

One of the most useful characteristics of χ^2 is that it consists of as many separable components as there are degrees of freedom, which, added together, make up respectively the χ^2 and the number of degrees of freedom for the entire series. Hence there will be as many added degrees of freedom for the new as compared with the original curve as there are additional observations, and the contribution to χ^2 corresponding to these additional degrees of freedom will measure the consistency of the new records with the old. The χ^2 for the original dosage-mortality curve is subtracted from the χ^2 for the pooled results, and the P corresponding to this difference is found in a table of χ^2 with the number of degrees of freedom equal to the number of new observations. If P is less than 0.05, the additional cases probably differ by more than the errors of sampling and are not sufficiently consistent with the original

experiments to warrant including them in a common dosage-mortality curve.

Ordinarily the same weights are used in computing both the original and the combined curves, so that the recalculation of the regression line required by this method is much less onerous than it seems at first sight. The estimates of χ^2 , however, are based primarily upon the variances used in these weights, which, in turn, have been computed from the provisional graphic curve of the original series. Ordinarily a small number of observations, which are near enough a given curve to warrant using the χ^2 test for their agreement, will not make a large enough change in the position of the regression line to invalidate the original weights if P is well above or below 0.05. In cases where P for the χ^2 of the difference is near the limit for conformity, the test cannot be considered a critical one until the weighting coefficients have been redetermined separately from the calculated regression line of the original records and of the original plus the additional records, preferably interpolating weighting coefficients for 0.01 of a probit. The curves are then recalculated and the difference in the two new χ^2 's will be a sufficiently accurate measure of agreement.

Indirect χ^2 tests for the discrepancy of a second curve. When the additional observations include two or more dosages, instead of comparing them with the standard as a miscellaneous group, they may first be tested for self-consistency by fitting them with a separate curve and the discrepancy between this second curve and the standard then measured by χ^2 . The standard dosage-mortality curve has been fitted by the usual procedure and, as stated, the χ^2 test has demonstrated a satisfactory agreement between observation and fitted curve. Subsequently a second regression line and χ^2 have been calculated from a new series of records, and again the agreement between observation and fitted curve is adequate. Do the two series differ by more than the errors of sampling? We may next pool the data of the two series and compute a third regression line from the combined record and apply the same χ^2 test (equation (7)) for the agreement of all observations with the combined curve. This value of χ^2 for the combined series is composed of three elements, the χ^2 representing the extent of variation of the observations in the standard series (I) about the line for Series I, a similar χ^2 for the variation of the observations in the new series (II) about its curve, and a χ^2 representing the discrepancy between these two regression lines. Hence the amount by which the χ^2 for the combined data exceeds the sum of the χ^2 's for the two original series is a χ^2 measure of the discrepancy.

The number of degrees of freedom, n , represented by this discrepancy is also secured by difference. For each curve, n is equal to the number of observations, n' , diminished by two—the degrees of freedom “used up” in fitting the curve. Since a separate treatment of the two series calls for fitting two curves with a total loss of four degrees of freedom, while pooling the same records requires a single curve with the loss of but two degrees of freedom, there is a gain of two in the latter procedure, representing the discrepancy.

Direct χ^2 tests for the discrepancy of a single observation. Instead of combining a new observation with a standard curve in order to measure its discrepancy, the χ^2 for the discrepancy may be determined directly from the data of the new observation and the constants for the original curve. When a single observed mortality in probits, y_1 , at a known dosage, x_1 , is to be compared with a given dosage-mortality curve,

$$\chi^2 = \frac{[y_1 - a - b(x_1 - \bar{x})]^2 Ag}{A + g(x_1 - \bar{x})^2} \quad \dots\dots(16),$$

and

$$g = \frac{w_1 S(w)}{w_1 + S(w)} \quad \dots\dots(17),$$

A (equation (6)) and $S(w)$ are constants from the standard curve, and w_1 is the weight (equation (1)) determined from the number of organisms in the new observation and the weighting coefficient at the probit value of the standard curve for the dosage x_1 . The expression within the brackets in the numerator will be recognised as the difference between the mortality in the additional observation and the expected mortality read from the regression line at this same dosage. When several additional observations are to be compared with a standard curve, especially if they are all at the same dosage, a χ^2 for the discrepancy of each case may be calculated from equation (16) and from their sum a combined χ^2 for the group, with as many degrees of freedom as there are separate determinations of mortality.

Direct χ^2 tests for the discrepancy of a second curve. Whenever a new series of observations includes two or more dosages, it is possible to fit an independent curve or regression line to the mortalities in the new series, and, as we have seen, this new curve will differ from the standard in two degrees of freedom. These two degrees of freedom, and the units of χ^2 to which they correspond, measure the difference between the curves of the two series due to a discrepancy in position, a , and a discrepancy in slope, b . When determined indirectly, these two contributions to χ^2 are not separable, so that their joint value is a measure of

the agreement between the two curves in both position and slope. The direct method of computation has the advantage of separating the two components in the discrepancy so that they are available for independent study when both the standard curve and the one for the new series have been determined from homogeneous data, as verified by equation (7). The following equations are based upon the same kind of reasoning as equation (7) and show the part of the discrepancy contributed by the difference between the two series in the parameter a and the part contributed by the difference in the parameter b :

$$b_c = \frac{[S(wxy) - \bar{x}S(wy)]_1 + [S(wxy) - \bar{x}S(wy)]_2}{A_1 + A_2} \quad \dots\dots(18),$$

$$\chi_a^2 = \frac{[(a_1 - a_2) - b_c(\bar{x}_1 - \bar{x}_2)]^2}{\frac{1}{S(w)_1} + \frac{1}{S(w)_2} + \frac{(\bar{x}_1 - \bar{x}_2)^2}{A_1 + A_2}} \quad \dots\dots(19),$$

and
$$\chi_b^2 = \frac{(b_1 - b_2)^2}{\frac{1}{A_1} + \frac{1}{A_2}} \quad \dots\dots(20),$$

when the subscripts 1 and 2 refer to the two series of records designated as the standard curve and the second curve. It can be demonstrated algebraically that $\chi_a^2 + \chi_b^2$ is exactly equal to the χ^2 for the two degrees of freedom $\chi_{1+2}^2 = \chi_1^2 + \chi_2^2$, so that, for example, χ_a^2 can be calculated by subtraction. The compound regression coefficient, b_c (equation (18)), represents the slope of the two parallel regression lines which fit the two series of observations most closely. Through its use, the second part of the numerator in equation (19) corrects for the fact that the respective values of a for the two series will usually be given at two different average dosages, \bar{x}_1 and \bar{x}_2 . χ_a^2 then becomes a test solely of difference in position without the bias of a possible discrepancy in slope. While χ_b^2 for a difference in slope does not require correction for the divergence in dosage, in cases where the entire range of mortalities cannot be fitted by a single straight line when dosage is plotted in logarithms, the two regression coefficients should apply to comparable sections of this range. In Fig. 3(15), for example, the dosage-mortality curve above a mortality at 4.56 probits has a significantly steeper slope than below this kill with χ_b^2 for the difference equal to 8.247. If the mortalities observed in Series I had all been greater than 4.56 probits or 33 per cent., and in Series II all below 33 per cent., a significant difference in slope would have quite a different meaning than when the two series fall within the same range of mortalities.

Even when computations are not carried beyond the determination of the regression line itself, one can estimate from equations (19) and (20) whether two given curves agree within the errors of sampling, but before a test for their mutual conformity can be fully valid, the dosage-mortality lines themselves should be tested by χ^2 for agreement with the observed mortalities. When a series consists of but two observations at two different dosages, its regression line will pass through both points and χ^2 for the discrepancy between the fitted curve and the observations will be 0 with 0 degrees of freedom. The two degrees of freedom representing the discrepancy in position and in slope between it and a second curve will still remain and these are best measured by χ_a^2 and χ_b^2 , calculated as before.

*Comparisons with a standard curve determined
from heterogeneous data.*

In actual experimentation, the data for calculating the standard curve is often subject to errors other than those due to sampling, especially when large numbers have so reduced the sampling error that it no longer conceals other sources of variation. When the separate lots of organisms for each dose are field-collected rather than taken from laboratory stocks of relatively uniform genetic composition, or when the age, sex, and physiological condition of all samples are not carefully controlled, or when a series of experiments requires several days or weeks to complete and the environment is not rigidly controlled, a completely homogeneous toxicological response cannot be expected. Yet many investigations are necessarily carried out under these or similar conditions, and if the different dosages have been applied in a random order, the curve determined from such heterogeneous data may still be sufficiently reliable to serve as a standard for many purposes. When the χ^2 test of equation (7) shows that the variation between the observations and the fitted line is in excess of random sampling, and a careful inspection of the plotted points reveals no trend which would justify substituting some function other than the logarithm of the dose for the abscissa, the regression may serve as a standard with the errors in its position and slope computed from the observed variation as given by equations (8) and (9) rather than from the theoretical or average sampling errors that were used in the preceding section. Instead of applying a χ^2 test, the difference in position or slope between the new observations and the standard is divided by the standard error of this difference and the

quotient, the statistic t , referred to Table IV in Fisher's text to determine its significance. The principle is the same as that used in dividing the difference between two averages by the standard error of the difference to secure an index to its significance. The exact form of the t test is dependent upon the number of observations in the new series.

The case of a single observation. The simplest case is that in which the observed mortality from a single experiment, in probits, is compared with the probit on the regression line at the same dosage. Since the expected kill, Y , as estimated from the curve, is calculated from equation (2), equation (2) may be substituted for Y in the formula for determining t . Then

$$t = \frac{y_1 - [a + b(x_1 - \bar{x})]}{\sqrt{\frac{\chi^2}{nw_1} + V(a) + V(b)(x_1 - \bar{x})^2}} = \frac{y_1 - a - b(x_1 - \bar{x})}{\sqrt{\frac{\chi^2}{n} \left\{ \frac{1}{w_1} + \frac{1}{S(w)} + \frac{(x_1 - \bar{x})^2}{A} \right\}}}$$

.....(21),

when y_1 is the observed mortality in probits in the new experiment determined at a dosage of x_1 . The standard error of the difference between the two expressions of the numerator is the square root of the sum of their variances, and the different elements in this total variance are given in the denominator in the same order as the terms in the numerator. The best estimate of the variance of y_1 is the ratio of the average variance about the standard curve, in terms of its χ^2 and degrees of freedom (n), to the weight, w_1 , of the new observation. This weight, in turn, is determined by equation (1) from the number of organisms in the new observation and the weighting coefficient at the probit value of the standard curve for the dosage x_1 . The variances in the two parameters, a and b , of the standard curve are derived from the actual variation about the curve as computed by equations (8) and (9). A value of t calculated in this way is entered in Table IV with the same number of degrees of freedom (n) as have been found in computing the standard curve. If P is less than 0.05, the new observation differs significantly from the standard curve; if more than 0.05, the new experiment agrees with the standard within the limits of observed variation.

The case of two observations. When observations at two different dosages are to be tested for agreement with a standard curve about which the variation, as measured by χ^2 , exceeds the error of sampling, the form of t will indicate agreement primarily in respect to position or primarily in respect to slope. If, when plotted on coordinate paper, the new observations are both on the same side of the standard curve, the

deviations will be of the same sign, either positive or negative, which suggests that they may be samples from a curve which differs from the standard in position. To test this possibility, the deviations are added in calculating t . For the two new observations, y_1 at x_1 and y_2 at x_2 , each having a separate t given by equation (21), we may add the two numerators and the two denominators beneath the square root sign to compute the combined t for the sum of their departures from the regression line. Combining terms, we have for a test primarily of agreement in position:

$$t_{\text{sum}} = \frac{y_1 + y_2 - \{2a + b(x_1 + x_2 - 2\bar{x})\}}{\sqrt{\frac{\chi^2}{nw_1} + \frac{\chi^2}{nw_2} + 4V(a) + V(b)(x_1 + x_2 - 2\bar{x})^2}} \quad \dots\dots(22).$$

When one of the new observations falls above the standard curve and one below it, one deviation will be positive and the other negative, and they will partially cancel one another when added algebraically as in equation (22), so that the value of t may be quite small. Yet the two tests may designate a significant change of slope in the new experiments. By subtracting these two deviations and combining terms, we have a test primarily for agreement in slope:

$$t_{\text{dif.}} = \frac{y_1 - y_2 - b(x_1 - x_2)}{\sqrt{\frac{\chi^2}{nw_1} + \frac{\chi^2}{nw_2} + V(b)(x_1 - x_2)^2}} \quad \dots\dots(23).$$

The same number of degrees of freedom applies to the t either for the sum or for the difference of the deviations, and is equal to the number of degrees of freedom about the standard curve.

The case of several observations. When three or more dosages are included in the new observations, they are not compared directly with the standard, but instead are fitted by the usual procedure with a regression line, which, in turn, is compared with the standard. If the χ^2 test (equation (7)) shows agreement between the new observations and their dosage-mortality regression line, the sampling errors in its position and slope are computed from equations (10) and (11); if not, the corresponding errors are those given by equations (8) and (9). In either case, the standard curve, which is here based on heterogeneous data, will, of course, have the errors in its position and slope determined from equations (8) and (9). The tests for agreement are simplified to determining whether two similar regression lines differ significantly in position and in slope by the application of the t test.

The value of t for testing the discrepancy in position is

$$t_a = \frac{(a_1 - a_2) - b_c (\bar{x}_1 - \bar{x}_2)}{\sqrt{V(a_1) + V(a_2) + (\bar{x}_1 - \bar{x}_2)^2 V(b_c)}} \quad \dots\dots(24),$$

when
$$V(b_c) = \frac{V(b_1) V(b_2)}{V(b_1) + V(b_2)} \quad \dots\dots(25),$$

a_1, a_2, \bar{x}_1 and \bar{x}_2 are determined from the two regression lines designated by the subscripts 1 and 2, b_c is the combined regression coefficient for the two curves computed by equation (18), and the variances in the denominator have been calculated as described. With n equal to the sum of the degrees of freedom in the two regression lines, the value for t_a is entered in Table IV of Fisher's text to find the corresponding P . If P is less than 0.05, the curve based on the later observations differs significantly from the standard in position; if more than 0.05, the two curves may be judged to have substantially the same position, or mean susceptibility to the poison, within the observed limits of variation.

The equivalent test for the discrepancy in slope, b , is made by entering the same t table with

$$t_b = \frac{b_1 - b_2}{\sqrt{V(b_1) + V(b_2)}} \quad \dots\dots(26).$$

Numerical examples.

For illustration, several of the above procedures may be applied to Strand's data on the effect of carbon disulphide upon *Tribolium*, which have been given in full in Table IV of the preceding paper.

In order to compare the several tests for the discrepancy of a single observation with a standard curve, we may use the range of higher dosages in Series I as our standard and assume that the only observation in Series II was that at 64.76 mg. of CS_2 per litre, a relatively discordant value which falls outside the zone of error in Fig. 3. Does it differ significantly from the curve established by Series I? The regression equation for Series I is $Y = 5.4743 + 23.784 (X - 1.7979)$. If this were the only information available concerning the standard curve, we would apply equation (13). At the log. dosage of 1.8113 (64.76 mg.), the mortality given by the standard curve is 5.7930 probits or 78.61 per cent. kill. Substituting in equation (13), $\chi^2 = \frac{(29 - 0.7861 \times 33)^2}{0.7861 \times 0.2139 \times 33} = 1.686$, and with $n = 1$, $P = 0.20$, or the observation from Series II does not differ significantly from the corresponding value on the standard curve determined from Series I, when the errors of this curve are disregarded. By the approximate formula (equation (14)), $\chi^2_{\text{approximate}} = (6.1700 - 5.7930)^2 \times 16.665 = 2.369$ and $P = 0.12$, still within the limits of sampling error.

Since the data for the standard Series I are available, the sampling errors of the curve should be taken into account. By means of equation (7), we find that χ^2 for the series is 0.811, $n = 2$ (since the expected survival from the two highest concentrations was only 0.66 of a beetle, these were lumped with the next lower concentration

in determining n'), and $P=0.67$, so that the variation in the data falls within the sampling error and the χ^2 tests for homogeneous data are applicable. The discrepancy between observation and curve may be derived first by the indirect method. A new curve which includes this additional observation and the records of Series I is computed and the χ^2 , determined (from equation (7)) for the scatter of all observations about it, is 2.613 with three degrees of freedom. Subtracting from this the χ^2 for the standard, we have for the additional observation $\chi^2=2.613-0.811=1.802$, $n=1$, and $P=0.18$. The agreement between the additional observation and the standard curve is satisfactory. The same χ^2 may be computed directly from equations (16) and (17),

when $g=\frac{16.67 \times 61.7}{16.67+61.7}=13.124$ and

$$\chi^2 = \frac{[6.160 - 5.474 - 23.784(1.8113 - 1.7979)]^2 \times 0.07201 \times 13.124}{0.07201 + 13.124(1.8113 - 1.7979)^2} = 1.806,$$

a result which agrees within the accuracy of the computation with that secured by the indirect method.

For illustrating the comparison of two curves, we may verify, within the range of the higher concentrations of CS_2 , the correspondence of the two series plotted in Fig. 3, experiments which were originally judged to be equivalent from inspection. Since χ^2 for the combined curve is within the limits of sampling error, the appropriate method for making the comparison is χ^2 rather than t . By the indirect approach, three curves are fitted, one to each series separately and one to the combination of the two series, and a χ^2 computed in each instance by equation (7). Since two degrees of freedom, representing the parameters for position and slope, are "lost" in fitting each curve, the number of degrees of freedom in the one curve fitted to the pooled data of Series I plus II will be two more than the sum of the degrees of freedom in the two curves fitted separately to Series I and to Series II. These two "extra" degrees of freedom and that part of χ^2 to which they correspond will represent the discrepancy between the regression lines fitted separately to Series I and to Series II. In the present instance, the χ^2 for this discrepancy is

$$5.5564 - (0.8107 + 4.3193) = 0.4264 \text{ with } n=2.$$

Instead of computing this indirectly, as a difference, we may split it up into its two components, the discrepancy in position and the discrepancy in slope, and, by direct methods, determine the χ^2 for these two discrepancies separately. Substituting in equations (18), (19) and (20),

$$b_o = \frac{1.7127 + 2.1093}{0.07201 + 0.07776} = 25.520,$$

$$\chi_a^2 = \frac{(5.4743 - 5.4274 - 25.520 \times 0.002465)^2}{0.01621 + 0.01488 + 0.000004} = 0.0082,$$

and

$$\chi_b^2 = \frac{(23.7840 - 27.1275)^2}{13.8869 + 12.8606} = 0.4180.$$

When the χ^2 's are tested separately, each with one degree of freedom, the two curves are seen to agree in position rather better than would ordinarily be expected ($P=0.93$), while in slope they show a common amount of divergence ($P=0.52$). The sum, $\chi_a^2 + \chi_b^2 = 0.4262$, is in close agreement with the 0.4264 determined by difference. A more exact correspondence cannot be expected because the weighting coefficients in this computation have all been based upon the single graphic line of Fig. 3. This source of error, however, is here obviously unimportant.

ESTIMATING DOSAGE FROM A STANDARD CURVE.

In the dosage-mortality curves which we have been describing and comparing, mortality, the dependent variable, has been plotted as a function of the dosage applied, the independent variable. In all instances the dosage was presumed to have been determined by exact experimental measurement and hence was not subject to the errors of sampling, which have proved so important in the estimation of mortality. The possibility has been considered by Iwaszkiewicz and Neyman⁽¹⁷⁾ from the mathematical standpoint that the number of effective "molecules" or other units of the poison in a single dose may be so small at the lower concentrations (or higher dilutions) as to involve a sampling error in the measurement of relative dosages. Since it is doubtful if this case will arise experimentally, we may consider the relative units of dosage in a given lot of toxic material as free of sampling error and accurate within the limits of the physical or chemical measurements involved. The problem, therefore, has been to estimate within known limits of accuracy the mortality which would result under the conditions of the experiment from any given dose of a specific poison. Tests for determining whether or not similar records of this type were in agreement have been considered with some care. The theoretical and practical objectives behind these toxicological experiments require that we consider next how this curve may be used in the reverse direction, so that the originally independent variate, the dosage, may be inferred from the dependent variate, the mortality.

Since the standard regression line (equation (2)) gives the most probable mortality in probits corresponding to any given dosage (in logarithms), it also gives the most probable dosage, X , corresponding to any required kill, Y . For convenience in computing dosage from mortality, equation (2) may be rearranged as follows:

$$X = \bar{x} + \frac{Y - a}{b} \quad \dots\dots(27),$$

when, as before, \bar{x} , a ($=\bar{y}$), and b are constants of the standard regression line. The accuracy with which such a dosage has been determined depends primarily upon whether the mortality, Y , has been selected by the experimenter and accordingly is itself free of error, or whether it has been obtained by experiment and is therefore subject to the fluctuations of sampling. In the first case, the accuracy in the estimated dosage is limited entirely by the error about the regression line that has been used as a standard, and can be determined by an extension of the equations

of the preceding paper. Such computations will be involved in comparing the relative susceptibilities of organisms to different toxic agents and in computing the dosage required for practical insect control. In the second case, the error in the estimated dosage is compounded of the known inaccuracy in the standard curve plus the sampling error inherent in the mortality of a particular sample or series of samples. The statistical procedures are necessarily somewhat more involved and are analogous to some of those given in the first part of the present paper. They apply primarily to the problem of biological assay, a subject that has been discussed so frequently in the recent literature that it is necessary here only to show the applicability of the present methods.

Determinations of dosage from a selected mortality.

In the preceding paper it was suggested that the individual lethal dose, the smallest dose of a toxic agent which just sufficed to kill a particular animal, could be considered as an index to its susceptibility. Similarly, the dosage which will kill any stated percentage of a population of animals may be considered as an index to the level of susceptibility in the population defined by that percentage. The most generally accepted index of susceptibility is the dosage killing 50 per cent., called the median lethal dose, or "LD50", a value that was adopted primarily because at this point the slope of the original sigmoid dosage-mortality curve is the steepest and its dosage can be interpolated with the greatest accuracy. However, when the curve is transformed into a straight line, this graphic restriction is no longer of importance, for the dosage at any other kill can be computed from the regression line with equal facility. But the accuracy with which any dosage can be inferred depends upon the width of the zone of error, such as that plotted in Fig. 3, and this is not equal over all parts of the curve. At the weighted average (in probits) of the observed mortalities it will be a minimum, for at this point the contribution of the error in the slope of the curve is zero. Since this average will vary from one regression line to the next, it is preferable to select for any series of standard curves some arbitrary mortality which will be near enough the average of all the series to minimise the errors of sampling. Both because it is near this average and is in widespread use, it is advantageous to select the 50 per cent. point or 5.0 probits. By means of the equation for the regression line and its error, the required dosages and their errors of determination may be computed, which will serve as exact indices both to the susceptibility of the organism and to the potency of the drug.

The median lethal dose will have greater advantages for physiological purposes than for comparing potential insecticides. The slope of the regression line sometimes varies with the same insect twofold or more between different poisons, so that their relative ranking at dosages killing 50 per cent. may not agree with that at dosages giving the high kills required for practical insect control. This possibility has been the only justification for those experiments in which treatments have been compared on the basis of kills of 100 per cent. Since the present methods permit an equally exact computation of the accuracy of the determination at any mortality within the range of dosages applied, it may be preferred to sacrifice a certain degree of accuracy in order to compare a series of compounds near the level of susceptibility in the insect population at which the most efficient would actually be used, such as at 7.0 probits (97.725 per cent. kill). The toxicological response represented by 6.0 probits is frequently as close to the general average of a series of tests as 5.0 probits and therefore will be nearly as precise, but it is near enough the region of practical application to be a good "compromise" value for the entomologist.

The minimal amount of poison which must reach each individual, to effect the mortality required for commercial insect control, can be estimated from the standard dosage-mortality regression line. The reliability of this procedure depends upon (1) whether the insects used in the toxicological test can be considered equivalent to samples from the infestation which must be treated and (2) the accuracy with which the curve has been determined by the samples actually used. Whether the experimental samples will be representative of the field population depends upon experimental conditions quite apart from the curve itself, but the accuracy with which the data have determined the curve can be computed with exactitude as has been shown. In Fig. 3, for example, the boundaries for odds of 19 to 1 are given, within which lies the "true" dosage-mortality relation in the insect population from which the samples were drawn. If, therefore, a given kill, such as 99 per cent., is decided upon as the objective for a particular control operation, the treatment should be gauged not by the most likely dosage corresponding to this mortality, which is given by the regression line at 7.3263 probits, but by the largest dosage which the true curve might show for this mortality within the odds of 19 to 1, and this is given by the lower of the two boundaries enclosing the true curve. The required dosage, therefore, is at the limit of error representing the largest resistance which might reasonably occur in the population at the mortality or degree of control

which is to be attempted, and the correction so obtained may be too important to be neglected with safety.

When the desired level of susceptibility or mortality has been selected, the limits within which the corresponding dosage has been established by the curve are computed by solving equation (12) for X at an assigned value of Y . Then

$$X = \bar{x} + \frac{b(Y-a)}{b^2 - t^2 V(b)} \pm \frac{t \sqrt{V(b)(Y-a)^2 + V(a)[b^2 - t^2 V(b)]}}{b^2 - t^2 V(b)} \quad \dots\dots(28).$$

The nature of the dosage-mortality regression will determine the values to be assigned to the variance in its position, $V(a)$, and slope, $V(b)$. If it were to have an absolute accuracy, these would be zero, and equation (28) would simplify to the form given in equation (27) for the single most probable dosage indicated by the regression line itself. If the χ^2 test (equation (7)) has shown that the errors of the standard curve are within its sampling limits, the general form of the variances would be used as computed from equations (10) and (11). Should the variation exceed that attributable to the errors of sampling, the observed variances in position and slope, given by equations (8) and (9), would be required.

It will be observed that the upper and lower limiting dosages are given by adding or subtracting the last term of equation (28) to the mid-value determined from the first two terms of the formula. This mid-value, however, does not coincide with the most probable value given by equation (26), but is displaced in the direction away from the mean, \bar{x} , by an amount proportional to the difference $(Y-a)$. Since the two branches of the hyperbola bounding the zone of error of the regression line are equidistant from that line in a vertical plane, they cannot be equidistant in the horizontal or dosage plane. Accordingly, the actual limits, in terms of original dosage units rather than of logarithms, will generally prove the most satisfactory form of statement. What these limits signify will depend upon the value assigned to the statistic t . If the familiar probable error is preferred, giving odds of 1 in 2 that the specified limits enclose the true dosage, t will be read from Fisher's Table IV at $P=0.5$ with n equal to the degrees of freedom about the curve. If limits at accepted odds for chance sampling variation of 1 in 20 are adopted, t will be taken from the same table at $P=0.05$.

Numerical example. As an example of the use of equation (28) in the determination of dosage for practical insect control, we may assume that a flour mill must be fumigated with carbon disulphide for the control of flour beetle. The dosage is to be so adjusted that each beetle will be exposed to an amount not less than that sufficient

to kill 99 per cent. of the individuals. We will assume further that the experimental samples in Table IV were representative of the same inherent level of and variation in susceptibility,¹ that they were exposed under the same temperature and humidity conditions as prevail in this mill, and that none of the younger stages are more resistant than the adult beetles which were used by Strand.² The problem is to estimate from the curve in Fig. 3 the minimal requirement after losses by leakage, absorption, poor diffusion, etc. have been deducted. The most probable value is the dosage given by the regression line, which may be calculated from equation (27) as follows:

$$X = 1.7967 + \frac{7.3263 - 5.4499}{25.5114} = 1.8703, \text{ or } 74.18 \text{ mg. CS}_2.$$

Although this is the most probable value, its use would allow no margin of safety if the position of the observed regression line should differ from that of the true regression line. Using the limit of error at a value of t for $P = 0.05$ corresponding to the larger dosage, which is the lower boundary in Fig. 3, and substituting in equation (27), we have

$$X = 1.7967 + \frac{25.5114(7.3263 - 5.4499)}{25.5114^2 - (2.365)^2 6.6683} \pm \frac{2.365 \sqrt{6.6683(1.8764)^2 + 0.007758 \times 613.534}}{613.534},$$

$$X = 1.8747 \pm 0.0205 = 1.8952, \text{ or } 78.56 \text{ mg. CS}_2.$$

The minimal requirement is seen to be 78.56 mg. of carbon disulphide per litre of air for five hours, as compared with 74.18 mg. if the possible error in the determination of the dosage-mortality curve had been neglected. While this correction may seem a small one, the rate of change in mortality per unit of fumigant is so large that, if the curve based upon 129 weight units were to differ from the true curve by the full amount indicated in the given odds, the number of survivors would be increased between four and five fold. For this reason, the use of the full equation for prediction purposes may have thoroughly practical advantages.

Methods of biological assay.

One of the most important uses of the dosage-mortality curve is for purposes of biological assay in the absence of an accurate or convenient physical or chemical method of analysis. Packard (18), for example, has found the mortality of *Drosophila* eggs a more reliable measure of the output of an X-ray tube than the customary commercial dosimeter and more convenient than the air ionisation chamber. In the pharmacological field, insulin and several other medicinal products are standardised primarily by toxicity tests on mice or other small mammals. Various procedures for such cases have been discussed in detail by Trevan (19),

¹ The importance of this qualification is emphasised by Shepard's letter to *Nature* referred to previously (15).

² Actually, Cotton (16) has shown that the larva and especially the pupa of *Tribolium* are more resistant to carbon disulphide than the adult. In the present case, therefore, the dosage-mortality curve should have been determined from pupae for practical use in estimating dosage rates.

Hemmingsen (6), Gaddum (5), and others, although many of these methods are now only of historical interest. We are here concerned solely in describing a procedure that is consistent with the foregoing analysis.

From its method of preparation or a preliminary chemical or physical test, a material that is to be assayed is assigned a provisional potency. By comparing its effectiveness with that of a standard preparation, this provisional potency is corrected or standardised. The procedure for such a biological assay will depend primarily upon whether the susceptibility of the test animals is sufficiently stable to permit a direct comparison with a well-established regression line for the standard preparation, or whether their susceptibility is so subject to unpredictable fluctuations from day to day that the comparison must depend upon parallel control tests with the standard.

Biological assays based directly upon a standard curve. In assays based directly upon a standard curve, a standard regression line with known errors in position and slope is established for a given stock of inbred laboratory animals, controlled conditions of treatment, and a standard preparation of the drug concerned. In an individual assay, the standard preparation is required as a control only at one or two concentrations to ensure that the susceptibility of the experimental animals has not changed. When the controls have been shown to agree with the standard regression line by the appropriate χ^2 or t test described in the first part of this paper, their present purpose is fulfilled and they may be set aside to be incorporated in future recalculations of the standard curve.

The estimation of the unknown in terms of the standard regression line is subject to two errors: (1) in the extent to which the mortality among the limited number of individuals treated with the unknown preparation approaches that which would be observed if the size of the sample or samples could be increased indefinitely, and (2) in the accuracy with which the standard curve describes the true relationship of dosage and mortality for the standard preparation. Of these two, the error in the unknown will be relatively greater than that in the standard curve, so that the comparison of the provisional and true dosages will be most accurate if it is made at the level of susceptibility at which the error in the unknown is a minimum. This will be at the average observed probit for the unknown, at which point only the sampling error in its position contributes to the inaccuracy of the unknown and not the error in slope. By subtracting from the average of the provisional dosage units (in logarithms) used in the assay, the logarithm dosage given by the standard

curve at the same probit, we obtain the logarithm of the ratio between the provisional and the standardised dosage unit. This ratio indicates by how much the provisional dosage unit must be multiplied to make it equivalent in potency to the standard. The procedure for a biological assay may be reduced, therefore, to the following steps:

(1) The results of a given assay, in terms of logarithms and probits for both the unknown and its control, are plotted with the standard regression line upon cross-section paper. The parameters of the standard line will have been computed previously and may be designated by the subscript "1".

(2) Agreement within the limits of sampling (or of the observed variation) between the control and the standard line is established by inspection, or, if not immediately apparent, by the methods of computation which have already been described. If it is not in agreement, the entire assay is repeated or the unknown is assayed in terms of the control by the methods used with animals which have a fluctuating susceptibility.

(3) If the assay is based upon a single test with the unknown, its parameters, x_2 , y_2 , and $V(a_2)$, will be respectively, the logarithm of the single provisional dosage, the probit of the observed percentage mortality, and $1/w_2$ when w_2 is computed from equation (1) for probit y_2 . If the assay is based upon more than one dosage of the unknown preparation, a provisional regression line parallel to the standard regression line is drawn through the plotted points of the unknown by inspection. This provisional regression line determines the weighting coefficient (Table III) and weight, w (equation (1)), to be assigned to each observation, so that the parameters for position, \bar{x}_2 and \bar{y}_2 , may be computed from equations (3) and (4). The variance in this position, $V(a_2)$, may be computed from equation (10) $\left(\frac{1}{S(w)} \right)$ if the standard curve has been calculated from homogeneous data; if the standard curve has been found by the χ^2 test (equation (7)) to be subject to variation other than the errors of sampling, $V(a_2)$ for the unknown will be larger than that given in equation (10) in a ratio approximately equal to $\frac{\chi^2}{n_1}$. Through the use of this ratio, which gives the average amount by which the variation in the standard experimental procedure exceeds that due to sampling, the variance in position can be estimated with a minimum of computation as

$$V(a_2) = \frac{1}{S(w_2)} \times \frac{\chi^2}{n_1}.$$

(4) The provisionally assigned potency of the material under assay is then standardised at the level of susceptibility represented by \bar{y}_2 . The difference between \bar{x}_2 and \bar{x}_1 at \bar{y}_2 , which may be designated as M (Gaddum's term), is the logarithm of the ratio between potencies of the two preparations. Substituting from equation (27),

$$M = \bar{x}_2 - \left(\bar{x}_1 + \frac{\bar{y}_2 - \bar{y}_1}{b_1} \right) \quad \text{.....(29),}$$

when \bar{x}_1 , \bar{y}_1 ($=a_1$), and b_1 are constants of the standard curve and \bar{x}_2 and \bar{y}_2 (or x_2 and y_2 if a single dosage) have been computed from the assay values with the unknown. The antilogarithm of M is the amount by which the provisionally assigned potency of the unknown must be multiplied to standardise it.

(5) The accuracy with which M has been determined may be computed from the following equation, which is similar to Gaddum's equation (10):

$$s_M = \frac{1}{b_1} \sqrt{V(a_2) + V(a_1) + \frac{(\bar{y}_2 - \bar{y}_1)^2 V(b_1)}{b_1^2}} \quad \text{.....(30).}$$

The variance in the position of the unknown, $V(a_2)$, is that described in the third paragraph above; the variances in the position and slope of the standard regression line are determined from equations (10) and (11) if the observations and the fitted curve agree within the limits of the χ^2 test (equation (7)), otherwise they are computed from equations (8) and (9). In order to utilise s_M to determine the limits within which M has been established for any given odds, it is multiplied by the constant t , which is read for the required value of P from Fisher's Table IV with n equal to the number of degrees of freedom in the standard curve, plus the number of separate determinations of mortality using the unknown, minus one. For the limits of error which might reasonably be regarded as safe (odds of 19 to 1), P will be 0.05, and the provisional potency of the unknown, multiplied by the antilogarithm of $M + ts_M$ and of $M - ts_M$ will give the highest and lowest standard potencies which might occur in that particular lot of the drug.

Some qualifications of the above procedure may be mentioned. Since the weight assigned to any observation is greatest at 50 per cent. kill, the errors of the final determination will be smallest if the provisional dosages are planned to cover the middle region of the curve. In many cases, however, the lower mortalities may not be consistent with the balance of the curve upon the convenient standard coordinates, and when the standard curve indicates a change in slope of this character, those observations should be omitted for which the provisional line,

fitted to determinations with the unknown, extends below the probit of the "break" in the standard. So far as this can be predetermined, it is advisable, therefore, to keep within the range of 35 and 85 per cent. kill. The method assumes not only that the level of susceptibility (the position of the standard line) but also that the relative variability within the population of test organisms (the slope) remains constant. If the plotted points seem to depart materially in slope from the standard curve, it is advisable to test whether this difference is significant, fitting the provisional curve for the unknown without reference to the slope of the standard. Should this prove to be the case, one more degree of freedom would be lost in applying equation (29), and the assay even then could not be accepted with the same confidence as if this indication of instability had not occurred.

Biological assays based upon parallel controls. A number of cases have been reported in which an unstable susceptibility to a drug has made it impossible to evaluate an unknown by direct comparison with a well-established standard curve. In some instances, as in insulin assay on white mice (6), the level of susceptibility (position) tends to fluctuate more than the relative susceptibilities within the population (slope). If the slope is substantially constant and can be derived from a previously determined standard regression line, it is possible to standardise an unknown from the results of a single dose of the unknown and a single dose of the standard preparation. The accuracy of such an assay, however, is so much less than in the case of several tests with each preparation that it does not merit further discussion. The preferred method of assay with an unstable susceptibility, therefore, is to test the standard preparation in parallel with the unknown at several dosages.

Provisional regression lines, approximately parallel, are fitted graphically to each series of points, and used in estimating the weight of each point. The mean positions of the two lines, \bar{x}_1, \bar{y}_1 and \bar{x}_2, \bar{y}_2 , and their compound regression coefficient, b_c (equation (18)), may then be computed. To determine the logarithm of the ratio of potencies, M is computed from equation (29), except that b_1 in the formula is replaced by b_c . The errors of sampling in the relatively small numbers employed for biological assays with small mammals, for example, ordinarily will mask any variation attributable to other factors in a perfected experimental procedure, although enough dosages should be used periodically to confirm an agreement within the error of sampling by the χ^2 test (equation (7)). Assuming that the agreement of the two series of records with their respective regression lines is adequate and that they do not differ signifi-

cantly in slope, the two variances in position may be calculated from equation (10) and that for the compound regression coefficient from equations (11) and (25). The error in the logarithm of the ratio of potencies, s_M , may be determined from these variances, replacing b_1 by b_c . In determining t , n will be equal to the total number of tests in the two series minus 3, representing the two parameters for position and the one parameter for the combined slope. If the assay is based upon only two observations each for the standard and the unknown, as is sometimes advocated, there will be only one degree of freedom in the determination of their ratio. By reference to Fisher's Table IV, it will be observed that in this case t will be relatively so large as to reduce the accuracy of the assay very materially. If, on the other hand, the same number of experimental animals are treated with a total of three concentrations each for the unknown and the standard, there will be three degrees of freedom left in the final assay, with a marked reduction in t and a corresponding increase in the accuracy of the estimate.

SUMMARY.

The theoretical and practical applications of the dosage-mortality curve usually involve either a comparison of similar series of records to determine whether they differ significantly, or the estimation of the dosage corresponding to a selected or observed mortality and the error of this estimate. In an earlier paper we have considered the methods appropriate for computing the dosage-mortality curve as a straight line and for measuring its error of estimation. The present paper is an extension of these methods to cover some of the more frequent applications of the curve.

In measuring the degree of agreement between different series of dosage-mortality data, it is convenient to refer to one series, which has been transformed into a straight regression line of known accuracy, as the standard, and to determine the agreement of similar or smaller groups of data with this standard. When the standard is of absolute accuracy, experimentally the exceptional case, only the second group of observations is subject to sampling error and the χ^2 test is applied in a form appropriate for the binomial distribution. When the standard curve has been determined from homogeneous data that involve no errors other than those of the random sampling of test animals which vary in their inherent susceptibility, two forms of the χ^2 test are available, both based primarily upon the weighting coefficients used in computing the dosage-mortality regression lines. In one case the additional observations are

combined with the original ones for a recomputation of the standard curve and of the χ^2 measure for the agreement of this second curve with the pooled data, the χ^2 for the discrepancy of the additional observations (or of their separately fitted curve) being derived by difference. In the second case the χ^2 's for these discrepancies are computed directly and, with two or more observations in the second series, separate the difference between the two series of data in position from that in slope. When the standard curve has been determined from heterogeneous data and the observed variation is statistically in excess of that attributable to errors of sampling, the χ^2 test is no longer applicable, and the discrepancy between the standard and one or more other observations is compared with the observed errors of estimation by means of the statistic t . Several of these procedures are illustrated by the numerical example taken from the preceding paper.

In expressing the relative susceptibilities of different biological races or species, or the relative potencies of toxic agents, the comparisons are in terms of dosages required to produce selected or observed mortalities rather than of mortalities produced by specified dosages. It is then important to measure the accuracy of such estimated dosages. When the mortality is chosen by the experimenter, the dosage estimated from the standard curve is subject only to the errors involved in its determination, and the relative merits of selecting different mortalities, such as at 5.0 and at 7.0 probits, as a basis for these comparisons is considered in the light of the use that is to be made of them. As an illustration, numerical data are given of one such application. When the mortality at which the dosages in a standard and a second series are to be related is determined by experiment, we have the conditions observed in the biological assay of drugs or other toxic materials. There is not only the error of the standard curve to be considered, but also the error in the mortality observed with the unknown which is being standardised. When the stock of test animals used in a particular biological assay is constant in respect to the standard preparation, both with regard to its average susceptibility and its relative susceptibility within the population, an unknown material can be standardised by direct comparison with a single curve of comparatively high accuracy, determined from many experiments with the standard preparation. When the population of test animals is not stable in these respects, each separate assay must be based upon parallel tests between the standard preparation and the unknown, and require, therefore, more observations to secure the same accuracy. A method is described for determining the ratio of potencies and the error of this ratio in both instances.

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ADDENDUM.

It is sometimes necessary to determine whether a series of regression lines, all of which agree with their respective observations within the sampling error, have the same slope. The χ^2 for the agreement of a series of regression coefficients is given by an extension of equation (20) as

$$\chi_b^2 = S \{A_1 (b_1 - b_e)^2\} \dots\dots(20a),$$

or the sum of as many terms, such as are included in the brackets following the summation sign (S), as there are independent estimates of b .

THE STANDARDISATION OF PETROLEUM AND TAR OILS AND PREPARATIONS AS INSECTICIDES¹

By HUBERT MARTIN, D.Sc., F.I.C.

(*Long Ashton Research Station, Bristol.*)

(With 1 Text-figure.)

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INTRODUCTORY.

THERE has in recent years been a growing and insistent demand on the part of users of insecticides and fungicides for some form of guarantee that the products shall reach a reasonable standard of efficiency and safety in use and further that materials sold under a given name shall not vary widely in quality from year to year. The problem of satisfying such requirements is not easy to solve, but it is evident that one method which would go some way towards improving the present situation would be to draw up detailed specifications for insecticides and fungicides in common use to which the manufacturers of these products would agree to conform, just as chemicals for use in analysis are made to the standards of purity laid down for "A.R." reagents, or drugs are prepared in accordance with the specifications of the British Pharmacopoeia.

This method has indeed already been adopted to some extent and agreed specifications, together with agreed methods of analysis, for some of the simpler materials in use, have lately been published by the Ministry of Agriculture (44). There still, however, remain many classes of insecticides and fungicides outside those dealt with in the publication referred to, and among these the various types of spray fluids containing tar and petroleum oils are perhaps the most important.

As a result of discussions at the Conference of Advisory Entomologists in December 1933, and further consideration of the matter by a small Committee appointed by the Conference, the writer undertook to draw up draft specifications and methods of analysis for the tar-distillate and mineral oil washes and mixed oil washes, and in the following paper, which is published at the suggestion of the Committee referred to, an attempt is made to show that the method of specification may be extended to include these materials. The suggestions are necessarily based on the published information on the subject at present available, and it has been thought necessary to include a rather full review of the evidence in their favour. The specifications are put forward in order that it may be ascertained how they work out in practice, and in the hope that they will receive consideration by those interested and perhaps serve as an encouragement to further experiment with properly analysed and controlled samples.

The standardisation of any insecticide or fungicide by analytical methods is the corollary of the successful elucidation of the chemical and physical properties which determine the biological properties and performance of the material under the conditions of use. The procedure adopted for the derivation of specifications suitable for the control of

petroleum and tar oils has accordingly been, firstly, an examination of the chemical and physical criteria used to define the oil, secondly, a survey of the results of scientific work upon the correlation of these criteria with biological properties, and, lastly, a coordination of these results to form the required specifications.

PETROLEUM OILS.

DEFINITION BY SPECIFICATION.

Petroleum oils are, chemically, complex mixtures of compounds in which hydrocarbons predominate and, as separation to individual compounds is impossible, an alternative method of defining a particular petroleum oil is necessary before any correlation between insecticidal properties and chemical composition can be attempted.

The crude oils were at first classified (1) according to the oil field from which they are produced, and (2) in American practice, into oils of paraffinic or asphaltic base according to the nature of the residuum (paraffin or asphalt) left by non-destructive distillation. Superimposed upon this older classification is a system, based on physical and chemical characteristics, which defines more closely the constituents of the oil. This system depends on the fact that, although the number of individual hydrocarbons present in an oil is high, they fall into more or less well-defined groups of compounds. Within such a group the hydrocarbons are of similar molecular constitution but differ in the number of carbon atoms present in the molecule, forming a series in which each member contains one $-\text{CH}_2-$ group more than its lower neighbour. In each group (or homologous series) the individual hydrocarbons are similar in those chemical properties determined by molecular constitution but show a graduation of those properties, mainly physical, which are determined by the size of molecule (molecular weight).

In the homologous series the boiling-points of the hydrocarbons increase as the series is ascended and distillation, which is the first step in the treatment of crude oils, results in a fractionation according to molecular weight, each fraction containing representatives of the different homologous series. A partial separation of the different homologous series is effected by the subsequent refinement processes.

The main homologous series now recognised as constituents of different petroleum oils may be placed into four broad groups:

(1) Paraffins: are characterised by chemical inertness and, in structure, regarded as saturated straight chain compounds.

(2) Naphthenes: resemble the paraffins in chemical properties, but, in structure, are branched cyclic compounds.

(3) Unsaturated hydrocarbons: are essentially straight chain compounds of greater chemical activity than the paraffins due to the presence of unsaturated linkages in the chain.

(4) Aromatic hydrocarbons: are cyclic compounds of greater chemical activity than the naphthenes. This greater activity is associated with the presence of the aromatic nucleus in their structure.

Refinement, either by sulphuric acid treatment or by the use of solvents such as liquid sulphur dioxide, results in the removal of hydrocarbons of the groups (3) and (4), leaving a greater proportion of the paraffins and naphthenes in the oil. In general, the more refined the oil the higher the content of hydrocarbons of groups (1) and (2).

GENERAL CONSIDERATIONS UPON THE SELECTION OF ITEMS OF SPECIFICATION.

This conception of petroleum oils as composed of groups of homologous series of hydrocarbons is the scientific basis underlying the use of the physical and chemical criteria employed, as items of specification, for the evaluation of the suitability of the oil for any particular industrial purpose. For the production of oils for specific industrial purposes from crudes the most important process is distillation, by which the oils are fractionated into such classes as the petrols, kerosenes and lubricating oils. The first petroleum oils to be used for insecticidal purposes were the kerosenes, but in later developments they have been displaced by lubricating oils. Kerosenes are nowadays used to some extent as solvents for contact insecticides, but their use as horticultural spray materials is limited and not specially considered here. The types of oils which are defined for insecticidal purposes by the proposed specifications are all of the lubricating oil class, and the criteria employed in the proposed specifications were primarily evolved for the determination of the suitability of an oil for specific lubricating purposes. It is convenient, therefore, before proceeding with a survey of the experimental data upon which the suggested specifications are based, to deal in a general manner with the criteria employed in these specifications. Obvious advantages accrue, if it be found possible to utilise criteria for which standard methods are already available and in routine use in the manufacture of lubricating oils.

From the conception of homologous series it is evident that these criteria may be divided into two broad groups:

(1) Those concerned with the physical properties and which indicate and are determined by the average molecular weight and complexity within the homologous series. Such criteria supply only secondary indications of the relative proportions of the groups of homologous series present.

(2) Those concerned with the chemical properties of the oil. These indicate and are determined by the relative proportions of the groups of homologous series present.

Further, it is to be expected that some degree of correlation will be found between the criteria within each of these two groups.

Considering first the physical criteria:

(a) *Distillation (boiling) range.* As has already been pointed out this item is of fundamental importance, but certain difficulties in its determination and interpretation have provoked suggestions that it may be replaced by other criteria. Further, de Ong⁽⁹⁾ considered that, as the distillation range gives indication of the properties of the oil at their boiling temperatures (300–400° C.), it can give little indication of the properties of the oil in the field at ordinary temperatures, and he proposed a volatility test to be used in its stead. Knight and Cleveland⁽³⁴⁾ advanced a similar objection to both boiling range and the percentage of oil distilling below a definite temperature (636° F.) in California, remarking that “we do not spray oils at 636° F.” Cunningham and Muggeridge⁽⁷⁾ also concluded that the determination of boiling range was unnecessary, as figures for viscosity and volatility give more reliable data by which the insecticidal value of an oil may be judged. It was, however, recognised by de Ong and by Cunningham and Muggeridge that the boiling range gives an indication of blending, such as the combination of a viscous oil of high boiling range with one of greater penetrating power such as kerosene, a combination protected for insecticidal purposes by U.S. Patent 1,914,903 (20. vi. 33). Smith⁽⁶¹⁾ concluded that distillation range provides a very dependable index to the volatility of oils and that it affords the most satisfactory basis for grading spray oils.

Secondly, it has been suggested that at the temperature of distillation, decomposition (pyrolysis, “cracking”) of the oil occurs. Pyrolysis does occur with resultant changes in the composition of the oil, but, at atmospheric pressure, the extent of the decomposition at temperatures below about 370° C. is small, and, as insecticidal tests show, insignificant. To reduce the extent of pyrolysis, Melander, Spuler and Green⁽⁴³⁾ employed distillation curves obtained at 40 mm. mercury pressure, but the

use of pressures lower than atmospheric has not become standard in petroleum technology, in which reproducible figures are obtained by the adoption of a standard technique in carrying out the determination.

Thirdly, it is difficult to express the results of the determination of boiling range in a manner which is not cumbersome. To give a single figure for the percentage of oil distilled below a certain temperature or to adopt Ostwald's suggestion⁽⁴⁸⁾ of an index number such as the mean of the temperatures at which 5, 10, 15, ..., 95 per cent. of the oil distils would appear inadequate, as much of the value of distillation range as a criterion of the oil (*e.g.* in the detection of blending) would be lost. A more satisfactory procedure would be to determine the minimum information as to boiling range that is adequate to characterise the oil for insecticidal purposes.

It is concluded therefore that the distillation range must appear in the specification to be adopted as the basis of the selection of petroleum oils as insecticides.

(b) *Volatility.* The conception behind the proposal mentioned above that the determination of distillation range may be replaced by a volatility test is that the insecticidal properties of an oil are determined by or related to the permanence of the oil film remaining after spraying. As the standard volatility tests adopted by the petroleum technologists are intended to give an indication of the permanence of oil films under the high temperature conditions at which the oil is required to act as a lubricant in steam and internal combustion engines, de Ong⁽⁹⁾ suggested the adoption of an evaporation test at 50–100° C. as approaching more closely the conditions in the field. His proposal has been approved by Swingle and Snapp⁽⁶⁷⁾ and by Cunningham and Muggeridge⁽⁷⁾. Dawsey⁽⁸⁾ criticised it as giving a distillation curve between 50 and 100° C., and suggested an alternative method in which the loss of weight of an oil film exposed under standard conditions at 100° F. is determined.

(c) *Flash and fire points.* These are the temperatures at which the oil, under standard conditions, gives a vapour capable of burning momentarily and continuously respectively. The use of these tests in petroleum technology is for the purpose of determining the fire hazard rather than the lubricating properties of the oil. Like the standard volatility tests the results give information regarding the lower boiling constituents of the oil and not the oil as a whole. Provided boiling range is given there seems no need to require these items in the specification of insecticidal oils.

(d) *Viscosity.* The petroleum technologist has not yet generally adopted absolute methods for determining viscosity but still relies upon

standard methods involving the time taken for a definite volume of oil to flow through a standard orifice at a given temperature. Unfortunately different standard methods have been adopted in various countries. Thus in the United States the Saybolt viscometer, in Europe the Engler viscometer, and in the British Empire the Redwood No. 1 viscometer are used. Moreover, different temperatures have been employed in experimental work upon the correlation of viscosity and the insecticidal performance of oils. It is generally agreed, however, that the temperature selected as suitable for the viscosity determination should be that used for routine work by the refiner which approaches nearest to the average outdoor temperature. In the United States the viscosity of oils used in insecticidal tests has been determined by the Saybolt viscometer at 100° F. In England, Redwood 1 viscosities at 70° F. were employed by Austin, Jary and Martin(1, 2, 3) in their work on oils for dormant application, whilst Tutin⁽⁶⁹⁾ adopted the same temperature for use with summer oils. Cunningham and Muggeridge⁽⁷⁾, however, advise the use of Redwood 1 at 100° F. under New Zealand conditions.

Now in every homologous series present in petroleum oils it has been found that viscosity increases with molecular weight and with boiling-point, and that there is a high degree of correlation between viscosity and boiling range. It might therefore be suggested that the viscosity figure is unnecessary if boiling range be given. The correlation is, however, affected by the relative proportions of the groups of homologous series present, and for this reason it would appear necessary on general lines to include both items in the specification. This secondary information given by the relationship between viscosity and boiling range is increased in value if viscosity figures over a range of temperature be stated, and the alternative of quoting viscosity at more than one temperature deserves consideration.

On the other hand, the practice, employed by the Californian citrus industry, of classifying oils into "light", "medium" and "heavy" groups, according to their viscosity, or the more correct terms "thin", "medium" and "thick", does not appear to be worth consideration unless these terms are ultimately defined by ranges of viscosity.

(e) *Pour and cold tests.* The temperatures at which, under standard conditions, the oil ceases to flow and becomes solid may give information as to whether the oil is of paraffin base (provided the wax has not previously been removed) or asphaltic base. Their use as a means of evaluating the insecticidal value of petroleum oils has been questioned by de Ong⁽⁹⁾ and by Swingle and Snapp⁽⁶⁷⁾, and there seems to be no

good reason for including these items in the specification of oils for insecticidal purposes.

(f) *Specific gravity.* Like viscosity the specific gravity of the oil is closely correlated with and increases with boiling range, but this correlation is affected by the relative proportions of the homologous groups of which the oil is made up. In general with oils of similar boiling range an increase of specific gravity indicates a higher proportion of carbon in the molecular composition, which is illustrated well by the fact that petroleum lubricating oils containing a preponderance of naphthenes have densities ranging from 0.85 to 0.95 at 60° F., whereas tar oils (which consist mainly of aromatic hydrocarbons) of similar or even slightly lower boiling range have specific gravities of approximately 1.10.

The inclusion of specific gravity in the specification of petroleum oils intended for horticultural purposes was considered unnecessary, provided viscosity and boiling range or volatility were given, by de Ong (9), Swingle and Snapp (67) and by Cunningham and Muggeridge (7). Because of the simplicity of determination and of its value in diagnosing the presence of material other than petroleum hydrocarbons, as in the case of tar-petroleum mixtures, specific gravity has, however, been retained in the specifications here suggested.

Turning now to the chemical criteria, the following have to be considered:

(g) *Base of oil.* Indicating the source of the crude oil from which the oil was manufactured. There is no specific test for determining this characteristic, but indirect information is afforded by the relationships between specific gravity, boiling range and viscosity or by the pour and cold tests.

(h) *Colour.* During refinement the colour of a petroleum oil disappears, the highly refined oil being colourless or "white" in the terms used by the refiner. Hence has arisen the distinction between "white" and "red" oils used in American horticulture, and the terms "water-white" and "half-white" applied in this country to oils of different degrees of refinement. Such a basis for defining the degree of refinement or the relative proportion of saturated hydrocarbons is obviously inadequate, especially as de Ong, Knight and Chamberlin (13) showed that filtration through Fuller's earth markedly reduced the colour of a lubricating oil without greatly altering the percentage of unsaturated hydrocarbons. de Ong (9), Cunningham and Muggeridge (7) and others have advised the discontinuance of the use of colour in the specification of petroleum oils to be used as insecticides.

(i) *Unsulphonated residue*. Indicates the percentage of oil remaining after treatment of the oil under standard conditions with sulphuric acid of definite strength. It gives a measure of the content of saturated hydrocarbons (paraffins and naphthenes), the unsaturated and aromatic compounds being removed by sulphonation or by polymerisation during the acid treatment. This test, which has been standardised by the (American) Association of Official Agricultural Chemists, has been widely used in America as an item for the evaluation of petroleum oils for spraying purposes, and no suggestion has yet been forthcoming from American workers that it should be replaced by other tests.

(j) *Iodine value*. This is based on the proportion of iodine absorbed by the oil under standard conditions, and it gives an indication of the relative number of unsaturated linkages in the molecular structure of the compounds present in the oil. The standard technique adopted for petroleum oils is that suggested by Wijs. This item has been suggested by Tutin⁽⁶⁹⁾ as more suitable than unsulphonated residue as an indication of the content of saturated hydrocarbons on the ground that it is more easily estimated. Cunningham and Muggeridge⁽⁷⁾ also preferred iodine value to a sulphonation figure.

(k) *Heat of bromination*. Resembles in purpose the iodine value determination but utilises a measurement of the heat evolved by the interaction of the oil with bromine as an index to the content of unsaturated hydrocarbons. This test was considered by Cunningham and Muggeridge to be more rapid than the Wijs iodine value estimation.

On general lines it is difficult to decide which of items (i), (j) or (k) should be recommended as the index to the content of saturated hydrocarbons. If a simpler but sufficiently accurate technique could be evolved for the determination of unsulphonated residue, this test would appear to be preferable, as it gives a direct approximation to the saturated hydrocarbon content.

(l) *Solubility in dimethyl sulphate*. Of various solubility tests which have been proposed as a means of determining the content of aromatic hydrocarbons, the Valenta test, in which dimethyl sulphate is used as the solvent, is the oldest and most widely used. Its greatest value is in the examination of tar-petroleum oil mixtures, but the possibility of its use with petroleum oils has to be considered.

(m) *Sludge test*. Petroleum oils, on exposure to high temperatures and to sunlight, may undergo oxidation with the production of acidic derivatives which, in lubrication, may contribute to sludge production. de Ong⁽⁹⁾ suggested that, as the acids formed by oxidation may cause

foliage damage, an oxidation or sludge test should be included in the examination of petroleum oils to be used as insecticides for application to foliage. Swingle and Snapp⁽⁶⁷⁾ pointed out that liability to oxidation is reduced by the removal of the more chemically reactive hydrocarbons and considered de Ong's proposal of doubtful value. Cunningham and Muggeridge⁽⁷⁾ also concluded that as foliage damage attributable to the oxidation of saturated petroleum oils had not been experimentally demonstrated and had been disregarded by recent workers, an oxidation test was probably of little significance in the selection of oils for insecticidal purposes. On general lines it would appear therefore that, if an item governing the content of saturated hydrocarbons be included, there is no need for an oxidation test.

(n) *Sulphur content.* De Ong⁽⁹⁾ also suggested that as the sulphur derivatives of petroleum hydrocarbons probably possess foliage-injuring properties, the sulphur content of petroleum oils for summer use should be stated.

These are the criteria to be considered in defining the type of oil suitable for insecticidal purposes, and there remains for general discussion the question of percentage of oil and the nature of the emulsifying agents to be used in the manufacture of petroleum oil preparations suitable for direct use by the grower. In the present spraying technique, water is the diluent normally used and, as is proverbially known, oil and water will not mix. For the preparation of oil sprays, additional ingredients, known generally as emulsifiers, are required to stabilise the dispersion of oil as suspended droplets so that the spray applied shall contain a uniform known amount of oil. The emulsifiers may conceivably influence the biological performance of the spray either by a direct modification of the insecticidal properties of the oil or by an indirect effect on the amount of oil retained by the sprayed surface. The latter effect has been found of importance, particularly in the use of petroleum oils as scalecides on citrus trees where the types of emulsion given by various emulsifiers have been roughly classified into quick-breaking and stable emulsions.

Only in the case of manufactured preparations from which the grower can prepare the spray merely by dilution with water in the spray tank is it necessary to consider the inclusion of items concerning type of emulsifier in the specification. Mention must, however, be made of those emulsification methods by which the sprays can be prepared from the oil itself by the grower, because these methods have been widely used in experimental work on the correlation of physical and chemical pro-

perties with the biological properties of oils. These methods for the home preparation of oil sprays fall into the following broad groups:

(i) *Tank mixture methods.* In these methods the oil, emulsifier and water are added direct to the spray tank, emulsification being achieved and maintained by vigorous mechanical agitation in tank and pumps. The emulsifiers used are many and include dried blood, skim milk and other casein preparations, and Bordeaux mixture. The emulsions obtained are generally unstable and quick breaking.

(ii) *Two-solution methods.* This process is best explained by an example: Oleic acid is dissolved in the oil and this solution poured into water containing sodium hydroxide. Interaction of the oleic acid and alkali gives a fatty acid soap which acts as the emulsifier, emulsification proceeding on gentle stirring of the mixture. Sulphonated fatty acids, etc., may be substituted for oleic acid, and the emulsions obtained by this method are in general stable and not quick breaking.

(iii) *Boiled soap methods.* This method involves the vigorous agitation of the oil in strong hot soap solutions. It is historically the first method to be used in England for insecticidal purposes and is still recommended in the United States for the home preparation of oil sprays. Fish-oil and resin soaps may be used, and the addition of materials such as cresylic acid (Richardson and Griffin⁽⁵⁶⁾) or certain alcohols (Whitcomb⁽⁷⁰⁾) has been suggested to facilitate the process. The soap emulsions in general are stable and yield, on dilution with soft water, emulsions which do not break quickly.

The commercial oil preparations which merely require dilution with water to be ready for use fall into two broad groups:

(i) *Miscible oils.* These are clear solutions of an emulsifier in the oil which on addition of water passes into emulsified form. The emulsifiers commonly used for such preparations are resin soaps, fatty acid soaps, sulphonated glyceride oils and beta-petroleum sulphonic acid soaps (Martin⁽³⁹⁾). Solution of the emulsifier in the oil may be aided by the addition of cresylic acid or alcohols. In general the emulsions obtained by the dilution of miscible oils are stable and not quick breaking.

(ii) *Stock emulsions.* These are concentrated emulsions manufactured by the processing of oil-water-emulsifier mixtures in an emulsifying mill. A wide range of emulsifiers may be used including glue, casein preparations, soaps, sulphite lye, gums, kaolin and combinations of these materials. The stability of the emulsions obtained by the dilution of such stock emulsions varies, but in certain cases, as when

casein ammonia or sulphite lye are used as emulsifiers, they are quick breaking.

Apart from the question of the influence of type of emulsification upon insecticidal properties the following practical points may be mentioned. Miscible oils usually contain but a small percentage of water and are accordingly more stable on exposure to frost and in storage than stock emulsions. Further, miscible oils are clear and usually limpid liquids readily poured and measured. Unfortunately they are, in common with all soap emulsions, easily broken when mixed with abnormally hard or saline waters or with other spray materials containing lime or magnesium salts. The stock emulsions are, in most cases, prepared with emulsifiers unaffected by calcium salts and are therefore more stable when diluted with hard water. With stock emulsions, however, creaming may occur on storage with consequent unevenness of oil distribution throughout the bulk. Moreover, the oil content of stock emulsions is often limited, for if too concentrated they become too viscous for easy mixing and measuring. It is therefore suggested that the type of emulsification, whether miscible oil or stock emulsion, should be specified.

BIOLOGICAL PERFORMANCE IN RELATION TO PHYSICAL AND CHEMICAL CHARACTERISTICS.

The needs of the citrus industry have played a great part in the development of lubricating oil insecticides, but the predominant use of petroleum oils in this country is against the pests of deciduous crops. For this purpose it is possible to make a broad division of the oils into those intended for use on the dormant plant and those for application to foliage. The former are conveniently termed winter washes, the latter summer washes, but there is, in practice, no clear-cut division. In the United States lubricating oil emulsions are often applied at the so-called delayed dormant stage when active bud development has begun, and, in this country, there is an increasing use of petroleum oils when and after the first leaves are visible.

The division into winter and summer washes facilitates the discussion of past work on the correlation of the physical and chemical characteristics of an oil with its insecticidal and phytocidal properties. The interrelationships shown by the various items within the physical (items (a)-(f)) and chemical ((g)-(n)) groups have already been indicated, and it is possible to deal with these two broad groups instead of taking each of the suggested items in turn. In deriving suitable specifications, attention will be given firstly to controlled experiments on the relationships

between characteristics and (i) insecticidal action, (ii) phytocidal properties, and, secondly, to specifications suggested by other authorities on the basis both of critical work and of practical experience of lubricating oil emulsions. These specifications will deal with oils for winter washes, for summer washes and for the intermediate bud-burst washes.

Winter washes.

The main purpose of the application of petroleum oils to dormant plants is to destroy hibernating insect pests of which the most important are those which over-winter in the egg stage, against which the oil functions as an ovicide, and scale insects.

(i) *Insecticidal action.*

(a) *Physical properties.*

By laboratory tests, employing the eggs of the capsid bug (*Lygus pabulinus*) laid in currant twigs, Austin, Jary and Martin^(1, 2, 3) showed that, with twenty oils of boiling range within the shaded area of Fig. 1, there was no correlation between ovicidal properties and boiling range. Further, oils of viscosities (Redwood 1 at 70° F.) ranging from 126 to 800 sec. and sp. gr. (60° F.) from 0.864 to 0.913, showed similar ovicidal properties. Indications were obtained in the 1932 trials of a tendency for oils of high viscosity to be less ovicidal, for the correlation coefficient for the eighteen petroleum oils examined was 0.392, which approaches the figure (0.468) requisite for significance. In the following years, however, in neither laboratory nor field trials were indications of this correlation obtained.

Against eggs of the fruit tree leaf-roller (*Cacoecia argyrospila*), Melander, Spuler and Green⁽⁴³⁾ showed that nine different oils of boiling range such that 50 per cent. distilled between 240 and 300° C. at 40 mm. absolute pressure had similar ovicidal properties. Harman⁽²⁴⁾ also found no difference in toxicity to leaf-roller eggs between a grade of oil (Red Engine) of viscosity 208–215 sec. and one (Diamond Paraffin) of viscosity 99 sec. Saybolt at 100° F.

Critical tests do not appear to have been carried out upon the action of petroleum oils on the eggs of red spider (*Oligonychus ulmi*), though there is evidence that with this test also toxicity is a property distributed over a wide range of lubricating oils. Thus, against the "European red mite" (*Paratetranychus pilosa* = *Oligonychus ulmi*), Hamilton⁽²²⁾ reported that most of the commercial miscible oils and oil emulsions available will give good control if properly applied, and later⁽²³⁾ he

concluded that thoroughness of application is more essential for the successful control of the mite than the kind of lubricating oil used.

Against San José scale (*Aspidiotus perniciosus*) Swingle and Snapp (67) reported the results of field tests on peach trees over a period of three years with sixteen different petroleum oils of characteristics given in Table I. From this table it will be seen that an inferior degree of control was given

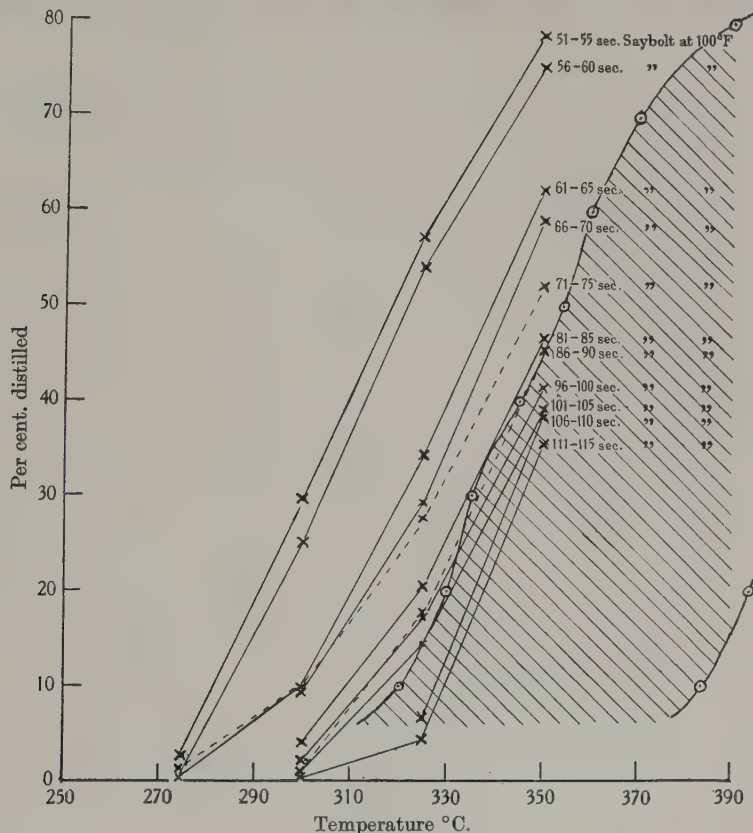


Fig. 1.

by oils of low viscosity. But oils above a certain minimum viscosity of approximately 125 sec. Saybolt at 100° F. or of volatility (loss during 4 hours at 105° C.) below 1.75 per cent., show similar scalecidal properties. With regard to the minimum viscosity, Swingle and Snapp, who carried out their tests in Georgia, pointed out that in more northerly States, oils of viscosity as low as 90 sec. Saybolt at 100° F. had been found effective, and suggested that the higher figure indicated by their results was asso-

ciated with the higher prevailing temperature. Quaintance, Newcomer and Porter⁽⁵³⁾ have also pointed out that the minimum effective viscosity varies in different parts of the United States, apparently because of climatic conditions, and that oils of viscosity above this minimum may be recommended for scale control. Spuler, Overley and Green⁽⁶³⁾ used, in experimental work in Washington, oils ranging in viscosity from 100 to 220 sec. Saybolt at 100° F. and obtained nearly 100 per cent. control within this range.

Table I.
*Experiments on the toxicity of various oils to the San José scale
on peach trees, Fort Valley, Georgia, 1926-9.*

Oil No.	Viscosity (Saybolt at 100° F.)	Density at 20° C. gm. per c.c.	Flash point ° F.	Fire point ° F.	Unsulphonated residue %	Volatility (4 hours at 105° C.) %	Apparent base	Percentage control obtained by dilutions of	
	sec.							2 %	3 %
1	47	0.847	276	300	74.0	4.18	Paraffin	—	0.0
2	76	0.873	325	365	68.8	1.50	„	—	96.33 ± 0.52
3	78	0.882	345	395	64.0	0.04	Intermediate	—	96.11 ± 3.30
4	79	0.887	349	414	62.0	0.93	„	91.4 ± 2.7	—
7	102	0.920	312	346	59.0	1.75	Naphthene	—	97.16 ± 0.95
9	105	0.884	365	420	64.8	0.60	Paraffin	—	96.88 ± 1.84
10	113	0.886	374	428	63.0	0.57	Intermediate	92.2 ± 2.4	—
12	124	0.889	365	412	60.0	1.21	Paraffin	93.8 ± 1.0	99.25 ± 0.40
13	137	0.924	325	360	59.0	0.08	Naphthene	—	99.12 ± 0.34
15	147	0.890	390	435	66.0	0.10	Paraffin	—	99.40 ± 0.20
18	168	0.895	404	451	61.0	0.60	Intermediate	94.57 ± 3.2	—
19	186	0.898	387	449	60.0	0.35	„	96.50 ± 1.0	99.25 ± 0.23
20	187	0.892	405	450	68.0	0.20	Paraffin	—	99.26 ± 0.24
21	200	0.895	422	474	62.0	0.22	„	97.70 ± 0.3	—
25	220	0.920	428	495	60.0	0.11	Intermediate	96.06 ± 0.9	—
33	308	0.904	440	495	51.0	0.06	Paraffin	—	99.55 ± 0.22

It will be noted from Table I that the flash and fire points of the petroleum oils used by Swingle and Snapp gave no indication of their toxicity to San José scale.

The experimental work in all cases indicates that, above a certain minimum viscosity, the insecticidal efficiency of the oils is independent of viscosity and, therefore, of those other physical characteristics correlated with viscosity. Because of the absence of boiling-range data in the American work quoted above, the average boiling ranges of oils of given Saybolt viscosity are indicated in Fig. 1.¹ It is apparent that insecticidal activity is independent of boiling range within wide limits.

(b) *Chemical properties.*

In their work with the capsid bug (*Lygus pabulinus*) Austin, Jary and Martin (2, 3) employed lubricating oils of paraffinic, naphthenic, asphaltic

¹ The curves are derived from the average boiling range and viscosity figures quoted by Marshall⁽³⁶⁾ for California oil sprays.

and Western base and found no difference in ovicidal properties dependent on base of oil. Against eggs of *Cacoecia argyrospila*, the earlier work of Penny⁽⁴⁹⁾ indicated that oils of paraffinic base were superior to those of naphthenic base, but no record is given, apart from Baumé gravity, of the physical characteristics of the oils used. From the gravity figures it appears possible that the failure of certain oils may be associated with their low boiling range. Swingle and Snapp⁽⁶⁷⁾, from the results quoted in Table I, concluded that the base of the oil has no apparent value as a measure of its toxicity.

The relationship between degree of refinement (content of saturated hydrocarbons) and ovicidal action was studied by Austin, Jary and Martin, who employed both iodine value and percentage of unsulphonated residue as indicators of the content of saturated hydrocarbons. With oils of iodine value from 0.4 to 10 and of percentage unsulphonated residue from 100 down to approximately 60, ovicidal efficiency was independent of degree of refinement. Evidence from trials with high-boiling tar oils, which had no unsulphonated residue, and with a sulphur dioxide soluble petroleum residue of low content of saturated hydrocarbons indicated a minimum of percentage unsulphonated residue below which the oil is of lower ovicidal efficiency. Melander, Spuler and Green reached a similar conclusion in their work upon the toxicity of petroleum oils to fruit tree leaf-roller eggs and stated that the additional refining beyond a certain minimum would represent expense without gain. In a later recommendation (Spuler, Overley and Green⁽⁶³⁾), based on four years' field trials, they stated that refinement beyond that represented by 50 per cent. unsulphonated residue was not necessary. Swingle and Snapp showed, as indicated in Table I, that, against San José scale, the percentage unsulphonated residue may vary greatly without differences in toxicity. A slight discrepancy is apparent between the results of these workers, who found an oil of no unsulphonated residue to be as effective as oils of 61 per cent. unsulphonated residue, and those of Austin, Jary and Martin, who showed oils of low unsulphonated residue to be less toxic to the eggs of *Lygus pabulinus* than oils of 60 per cent. or greater unsulphonated residue. The oil (No. 19, Table I) used by Swingle and Snapp would appear, however, to be abnormal in that its density is unusually low for a high-boiling petroleum oil with so small a content of saturated hydrocarbons.

The general conclusion from controlled experiments is, therefore, that the insecticidal efficiency of oils for use in the dormant season is independent of the degree of refinement above a certain minimum. It would

appear superfluous to require specific mention of the percentage of sulphur or the liability to oxidation in the description of oils for dormant washes, as both figures will be controlled sufficiently by the degree of refinement.

(c) *Oil content.*

It has generally been regarded as axiomatic that the efficiency of an oil spray for any given oil will increase normally with its oil content. Experimental tests by Austin, Jary and Martin⁽³⁾ confirmed this view.

(d) *Type of emulsification.*

In laboratory and field trials, Austin, Jary and Martin⁽²⁾ compared stable two-solution oleic acid emulsions with quick-breaking Bordeaux mixture emulsions and could detect no difference in ovicidal efficiency against the capsids, *Lygus pabulinus* and *Plesiocoris rugicollis*, and as general winter washes. In further details, given by Steer⁽⁶⁶⁾, a similar control of *P. rugicollis* was obtained with oleic acid and Bordeaux mixture emulsions of a combined tar-petroleum oil.

Melander, Spuler and Green⁽⁴³⁾, who used a cresylic acid potash fish-oil emulsifier, reported that the addition of excess of the emulsifier did not affect the toxicity of the wash to leaf-roller eggs or to San José scale. They stated, however, that the addition of a casein lime spreader to a miscible oil spray decreased its insecticidal efficiency, though this spreader could be added without effect to oil sprays prepared with a casein emulsifier. It may be suggested, though these authors did not mention the possibility, that in the case of the miscible oil-casein lime combination, the reduction of efficiency was associated with an interaction of the lime and the fatty acid soaps presumably present in the miscible oil which renders the mixture chemically incompatible and not to be recommended to growers.

Swingle and Snapp examined the action of sprays of equal oil concentration prepared with different emulsifiers upon San José scale and found no correlation between insecticidal efficiency and the average diameter of the oil droplets of the emulsions (which in general indicates the stability of emulsions). They concluded that the control obtained with a quick-breaking emulsion was slightly better than that secured with emulsions of a very stable type, but no better than the average results obtained, over a seven-year period, with the Government standard boiled emulsion (potash fish-oil soap as emulsifier) of the same oil concentration. Spuler, Overley and Green⁽⁶³⁾ also found that quick-breaking emulsions gave a better control of scale insects than stable emulsions

when applied in the laboratory, but that this difference was not obtained in the field. They advanced as explanation the possibility that in the laboratory the insects were uniformly oversprayed, and concluded that the emulsifier of the oil was not an important factor in insect control under field conditions. Newcomer and Yothers (47) stated that similar efficiencies were shown by unstable casein and stable boiled soap emulsions and by miscible oils against San José scale.

Critical work would thus indicate that there is a remote possibility that type of emulsification or emulsifier may affect the insecticidal efficiency of dormant oil sprays, but, as will be seen in the discussion of the general recommendations, this possibility may be disregarded in preparing specifications for the control of commercial winter petroleum oil preparations.

(ii) *Phytocidal action.*

(a) *Physical properties.*

No critical work appears to have been carried out on the influence of the physical properties of oils upon the extent of bud damage by winter petroleum washes. This of itself may be good evidence that bud damage is not of importance. Quaintance, Newcomer and Porter (53), for example, stated that "the extensive use of many kinds of oil emulsions under all conceivable weather conditions, warrants the statement that there is little danger from the use of properly prepared oil emulsions or miscible oils when the trees are fully dormant". There is a tendency, in reported cases of bud damage, to associate injury with oil concentration rather than with the characteristics of the oil.

On the other hand, with oil sprays applied at the delayed dormant and later stages, there are indications that phytocidal action can be associated with the type of oil and emulsion and ceases to be governed solely by the concentration of oil applied. Thus Ross (59) reported greater injury to apples by sprays containing oils of viscosity 85–95 sec. than with oils of 170–220 sec. Saybolt at 100° F.

(b) *Chemical properties.*

Green (20) reported that, although it has been claimed that refined oils applied in dormant washes cause less injury than crude oils, no observation of appreciable amount of oil injury had been obtained. With oil sprays applied after bud development had become visible, there is evidence that oil injury is associated with degree of refinement. Thus Swingle and Snapp found that, with oil sprays applied to peach trees

after bud burst, a high percentage unsulphonated residue indicated low toxicity to leaves and blossom.

Further discussion of the correlation of phytocidal properties and degree of refinement of petroleum oils for use at stages when the plant is not dormant will be delayed until the consideration of summer washes. For use on the dormant plant there is no evidence that oils of a degree of refinement above that represented by a minimum percentage of unsulphonated residue are inferior to highly refined oils on the score of liability to cause injury.

(c) *Concentration of oil.*

As has already been mentioned, the oil concentration of winter petroleum washes appears to be more important, from the phytocidal aspect, than the quality of the oil used.

(d) *Type of emulsification.*

Austin, Jary and Martin⁽²⁾ and Steer⁽⁶⁶⁾, in field trials with washes containing a combined tar-petroleum oil, obtained bud damage to apples which was similar with stable oleic acid and unstable Bordeaux mixture emulsions. With delayed dormant washes, on the other hand, Spuler, Overley and Green⁽⁶³⁾ found that, at a critical stage of development of apple buds, greater damage was caused by quick-breaking emulsions than by miscible oils (which yield stable emulsions). This is a second illustration of the need of a separate discussion of the properties of oil preparations for use at stages later than the dormant.

(iii) *Specifications suggested elsewhere.*

From the results of comparative trials and from general field experience of lubricating oil emulsions, specifications for dormant oils and oil preparations have been suggested recently by authorities in other countries. To enable a comparison of these specifications, they have been tabulated under physical and chemical criteria to form Tables II and III.

In every instance, the striking feature of the specifications summarised in Tables II and III is the wide range of oils recommended as equally effective in winter washes.

(iv) *Conclusions.*

It is now possible to deduce the required specifications for oil and oil preparations upon the basis of the results of controlled work and general practical experience, reviewing in turn the items suggested above:

(a) *Boiling range.* (b) *Volatility.* The majority of previous investigators have preferred a volatility test, but it is suggested that, in view

Table II.
Physical criteria.

Authority	Locality	Boiling range	Volatility	Viscosity	Sp. gr.
Reports on Oil Emulsions (54)	U.S.A. (general)	—	<2 %	90-250 sec. Saybolt at 100° F.	0.87-0.93 (20° C.)
„	U.S.A. (scale insects)	—	<1 %	180-220 sec. Saybolt at 100° F.	0.88-0.91 (20° C.)
De Ong (9)	California	—	—	85-120 sec. Saybolt at 100° F.	—
English (15)	Illinois (scale insects)	—	Loss at 212° F. for 8 hours ‡ 1 %	‡ 80 sec. Saybolt at 100° F.	—
Ross (59)	Ontario	—	Loss at 105-110° C. after 4 hours <0.41 %	170-220 sec. Saybolt at 100° F.	—
Crosby, Mills and Blauvelt (6)	New York State	—	Loss at 220-230° C. after 4 hours ‡ 2 %	90-215 sec. Saybolt at 100° F.	0.88-0.90 (20° C.)
Swingle and Snapp (67)	Southern States of U.S.A.	—	Loss at 105° C. after 4 hours ‡ 1.75 %	‡ 125 sec. Saybolt at 100° F.	Unimportant
Quaintance, Newcomer and Porter (53)	Arkansas Northern U.S.A. S. and E. States of U.S.A.	— — —	Should be low	Approx. 200 sec. Saybolt at 100° F.	—
				>125 sec. Saybolt at 100° F.	—
				>90-100 sec. Saybolt at 100° F.	—
Spuler, Overlay and Green (63)	Washington	—	—	100-220 sec. Saybolt at 100° F.	—
Cunningham and Muggeridge (7)	New Zealand	—	Should be very low	‡ 85 sec. and preferably >125 sec. Redwood 1 at 100° F.	—
<i>Bull. Ohio agric. Exp. Sta.</i> No. 128 (1934)	Ohio	—	<2 %	90-250 sec. Saybolt at 100° F.	0.88-0.91 (20° C.)

— = no mention; < = less than; > = greater than; ‡ = not less than; ‡ = not greater than.

of its fundamental nature and diagnostic value, boiling range should be substituted. For reasons given in the discussion of the analytical methods (p. 401) it seems sufficient to quote three temperatures to define the boiling range, *e.g.* 90 per cent. to distil above 315° C., 50 per cent. above 350° C. and 20 per cent. above 380° C. The volatility of an oil with this boiling range will fall within the limits suggested by the American workers.

(c) *Flash and fire points.* May be neglected.

(d) *Viscosity.* The minimum viscosity figure is determined mainly by the boiling range suggested and it would appear that 125 sec. Redwood 1 at 70° F. is suitable. As has been pointed out above, the minimum effective viscosity is dependent on prevailing temperature of the locality where the spray is to be applied. Assuming that the temperature conditions of the fruit-growing areas of the North Atlantic States of America

Table III.
Chemical criteria.

Authority	Locality	Base of oil	% unsulphonated residue	Iodine value	Oil content of commercial preparations	Type of emulsifier
Reports on Oil Emulsions (54)	U.S.A.	—	—	—	—	Boiled soap or cold mix (Bordeaux, lime casein, etc.) equal
De Ong (9)	California	—	>65–75 %	—	—	—
Crosby, Mills and Blauvelt (6)	New York State	—	—	—	Should be stated (usually about 66 %)	Recommend boiled soap or Bordeaux emulsions
Swingle and Snapp (67)	Southern States of U.S.A.	Without effect	Without effect	—	Official limit in Georgia $\pm 66\%$ by vol. (20° C.)	Unstable type slightly superior but difference slight
Quaintance, Newcomer and Porter (53)	U.S.A.	Of no special importance	$\pm 50\%$	—	—	Soap, lime casein and Bordeaux emulsions recommended
Spuler, Overley and Green (63)	Washington	—	$\pm 50\%$	—	—	Unimportant
<i>Bull. Va agric. Exp. Sta.</i> No. 126 (1932)	Virginia	—	Recommends miscellaneous oils	—	—	Recommends sulphite lye and lime casein as emulsifiers
Young and Morris (74)	U.S.A.	—	$\pm 55\%$, 60–70 % preferable	—	—	—
Cunningham and Muggerridge (7)	New Zealand	—	$\pm 65\%$	≥ 30	—	Miscible oils or boiled soap emulsions

are equivalent to those of England during the dormant spraying season and interpreting into Saybolt viscosity the Redwood 1 figure by means of the boiling-range data supplied by Marshall (36), the suggested minimum Redwood viscosity of 125 sec. at 70° F. corresponds approximately to 95 sec. Saybolt at 100° F., which agrees well with the minima recorded in Table II.

The maximum figure for viscosity is dependent, not on insecticidal factors but on ease of handling by the grower. A viscous oil becomes difficult to measure and, when used for commercial preparations, the percentage of oil which can be incorporated in a stock emulsion type of preparation which can be poured and measured easily, decreases as the viscosity increases. On the basis of Austin, Jary and Martin's work, it is suggested that the maximum viscosity should be 500 sec. Redwood 1 at 70° F. It would appear unnecessary, in view of the wide specification, to require the statement of viscosity at more than one temperature.

(e) *Pour and cold tests.* May be neglected.

(f) *Specific gravity.* Although of little use in defining the insecticidal properties of petroleum oils for dormant use, it is suggested that the specific gravity be retained in the specification because of its diagnostic

value. The limits are fixed by the selected boiling range and viscosity figures at 0.86–0.92 at 60° F.

(g) *Base of oil.* (h) *Colour.* May be neglected.

(i) *Unsulphonated residue.* A minimum percentage by volume unsulphonated residue of 60 per cent., when determined by the technique described in the analytical details, is suggested on the basis of Austin, Jary and Martin's work. This minimum is in good agreement with those suggested by other authorities (see Table III) and permits the use of the cheaper grades of spindle and lubricating oils.

(j) *Iodine value.* (k) *Heat of bromination.* May be neglected if (i) be given.

(l) *Solubility in dimethyl sulphate.* The content of aromatic hydrocarbons of straight petroleum lubricating oils is normally small, and it has been shown by Austin, Jary and Martin(2) that the addition of aromatics (as tar oils) to petroleum oils does not reduce ovicidal efficiency. It would therefore appear unnecessary to include this item in the specification of petroleum oils provided that the figure for unsulphonated residue be included.

(m) *Sludge test.* (n) *Sulphur content.* In view of the relatively high content of unsaturated hydrocarbons permitted by the unsulphonated residue figure, it would appear superfluous to require either of these items in the specification.

The full specification is therefore:

Specific gravity (60° F.), 0.86–0.92.

Boiling range:

At least 90 per cent. by volume to distil above 315° C.

„ 50 per cent. „ 350° C.

„ 20 per cent. „ 380° C.

Viscosity: Redwood 1 at 70° F.:

Not less than 125 sec. and not greater than 500 sec.

Unsulphonated residue:

Not less than 60 per cent. by volume.

Further, the oil should yield 100 per cent. neutral oils and, for a reason given on p. 402, be free from alkali when tested by the methods prescribed.

It is evident that a specification of this complexity is a description not well suited for easy reference by the grower. For his convenience an oil fulfilling the above requirements is therefore called a Grade E oil.

In the case of manufactured oil preparations which have merely to be diluted by the grower to give the required spray, two further items require consideration.

(o) *Oil content.* The percentage of oil which can be conveniently incorporated in the oil preparation varies according to the type of product and the emulsifier used. In general, the miscible oil type can be prepared with a higher oil content than the stock emulsion type and it thus becomes necessary to suggest different minimum oil contents for the two types of product.

(p) *Types of emulsifier.* The results of critical and general work upon the dormant petroleum washes show that insecticidal efficiency is independent of type of emulsifier. Nor, as with summer washes, is it usual or as yet shown to be advantageous to incorporate other insecticidal or fungicidal materials in the dormant wash. It is therefore unnecessary to specify, apart from the distinction between the miscible oils and stock emulsions, the emulsifier or type of emulsification.

The oil content remains for consideration and it is suggested that the following minimum figures for percentage oil by weight be adopted:

(1) Miscible oil type. Not less than 80 per cent. by weight neutral hydrocarbons.

(2) Stock emulsion type:

 Type I: not less than 80 per cent. by weight neutral hydrocarbons;

 Type II: not less than 66.7 per cent. by weight neutral hydrocarbons;

determined in all cases by the prescribed analytical methods.

Summer washes.

(i) *Insecticidal action.*

(a) *Physical properties.*

The pioneer work on citrus of de Ong, Knight and Chamberlin⁽¹³⁾ indicated that the insecticidal action of petroleum oils is dependent on the permanence of the oil film left after spraying and, in the case of scale insects, upon the ability of the insect to expel oils of viscosity below a certain minimum from its tracheal system. From laboratory tests against citrus red scale (*Chrysomphalus aurantii*) these investigators concluded that "oils, with a rather wide range of high viscosity, other things being equal, gave complete control. Below the minimum of this range, the lighter the oil the less certain will be the kill." Unfortunately they employed arbitrary viscosity figures; but two oils, one of viscosity 330–340 sec. and the other of 106 sec. Saybolt at 100° F., gave complete control at 2 per cent. when applied in unstable lime casein emulsions.

Table IV, compiled from data given by de Ong, Knight and Chamberlin, illustrates the inefficiency of a light lubricating oil and a kerosene. These workers also stated that oils of extremely high viscosity are likewise ineffective, but this conclusion appears to have been based on results with castor oil which, being a glyceride oil and hence of different molecular structure, may not be comparable with petroleum oils. The more recent work on citrus pests of Ebeling⁽¹⁴⁾ also indicated that, against red scale, insecticidal efficiency is dependent on the "heaviness" of the oil, employing heaviness as synonymous with high viscosity; and Smith⁽⁶¹⁾, from controlled field and laboratory trials, summarised in Table V, concluded that the most effective oil for the control of citrus scale insects is the heaviest, employing heaviness as indicating high boiling range.

Table IV.

Toxicity of petroleum oils to red scale, adopted from de Ong, Knight and Chamberlin⁽¹³⁾.

Oil No.	Oil description	Sp. gr.	Viscosity		Sulphur %	Unsulphonated residue %	Oil in emulsions %	Scale surviving test %
			Saybolt at 100° F. sec.	Arbitrary				
—	Castor oil	—	—	1840	—	—	2	48.0
6	Heavy lubricating oil	—	330-340	364	0.6	58	2	0.0
5	Oronite crystal oil	0.881	106	100	0.006	98	2	0.0
—	Light lubricating oil	—	—	38	—	—	2	2.0
4a	Refined kerosene	0.814	—	21+	0.006	98	20	19.0

Table V.

Control of citrus scales by petroleum oils. Compiled from Smith⁽⁶¹⁾.

Oil content of spray (%)...		2.5		2.0		1.0	
Viscosity (Saybolt at 100° F.) (sec.)		100	80	100	80	75	50
Percentage scale insects alive							
<i>Citricola</i> scale		—	—	—	—	1.36	4.21
"		—	—	—	—	1.70	0.09
"	(av. from misc. trials)	—	—	—	—	1.58	2.26
Black scale		—	—	—	—	0.34	1.05
Red scale		—	—	13.13	15.29	—	—
"		7.71	7.12	17.43	28.00	—	—
"		4.20	12.83	13.49	18.72	—	—
"		3.23	10.87	6.31	13.22	—	—
"		9.03	7.53	26.92	11.84	—	—
"		7.21	9.91	14.58	17.89	—	—
"		2.81	8.26	5.39	14.05	—	—
"		14.03	11.36	21.79	32.59	—	—
"		—	—	5.95	15.76	—	—
"	(av. field trials)	5.79	9.75	14.48	19.80	—	—
"	(av. bark trials)	8.20	13.70	20.60	19.40	—	—

Against pests of deciduous and herbaceous plants, the action of petroleum oils was critically examined by Griffin, Richardson and Burdette⁽²¹⁾ employing *Aphis rumicis* as test organism. Their results, summarised in Table VI, showed that the insecticidal efficiency of oils applied under similar conditions of emulsification is independent of viscosity when above a certain minimum figure, and that the toxicity of the sprays was uninfluenced by the physical characteristics of oils of the lubricating type. Sprays containing oils (kerosene and gas oil) more volatile than lubricating oils required two or more times the concentration of oil to produce the same effect as the lubricating oil emulsions. There is, however, evidence from other sources that, among the lubricating oils, toxicity may increase with viscosity or boiling range. Spuler, Overley and Green⁽⁶⁴⁾, for example, tested oils of unsulphonated residue greater than 85 per cent. upon codling moth (*Carpocapsa pomonella*) and obtained results given in Table VII.

Table VI.
Toxicity of petroleum oils to Aphis rumicis, compiled from Griffin, Richardson and Burdette (21).

Oil	Sp. gr. 20° C.	Flash point ° F.	Fire point ° F.	Volatility 4 hours at 105° C. %	Viscosity Saybolt at 100° F. sec.	Unsulphonated residue %	Concentration %	Percentage killed	
								Potash-fish oil emulsions (hot 40 lb. pressure)	Potash-fish oil + cresol emulsions (cold- stirred)
Kerosene	0.811	130	150	58.2	31	83	$\begin{cases} 1.0 \\ 2.5 \end{cases}$	96.7 ± 3.60	41.9 ± 15.98
Gas oil	0.879	170	205	23.3	50	64	$\begin{cases} 0.5 \\ 1.0 \end{cases}$	99.2 ± 0.35	89.7 ± 6.57
Liquid petrolatum	0.879	390	425	0.2	218	97	0.5	85.3 ± 4.78	—
Paraffin 115	0.883	360	400	0.4	110	67	0.5	98.5 ± 0.73	54.7 ± 6.71
Paraffin 222	0.896	380	425	0.3	218	68	0.5	97.3 ± 1.68	61.5 ± 12.94
Paraffin 318	0.897	415	475	0.06	313	76	0.5	99.2 ± 0.95	77.8 ± 1.87
Naphthene 100	0.912	305	335	1.6	105	63	0.5	97.0 ± 2.14	57.2 ± 13.80
Naphthene 202	0.919	335	365	0.7	200	60	0.5	98.0 ± 1.30	55.7 ± 7.52
Naphthene 305	0.924	345	380	0.6	298	60	0.5	98.6 ± 1.25	65.6 ± 6.13
								97.5 ± 2.07	60.3 ± 8.54
								98.7 ± 0.59	61.0 ± 5.25

Table VII.
Influence of viscosity on insecticidal value of oil (from Spuler, Overley and Green (64)).

Test No.	Viscosity 50–55 sec. (Saybolt at 100° F.)		Viscosity 70–75 sec. (Saybolt at 100° F.)		Viscosity 110–120 sec. (Saybolt at 100° F.)	
	Total fruit	Percentage wormy	Total fruit	Percentage wormy	Total fruit	Percentage wormy
1	7,745	10.92	—	—	5,591	6.06
2	11,688	9.12	—	—	13,405	4.55
3	5,008	23.73	—	—	5,038	7.74
4	6,119	12.42	—	—	5,488	7.10
5	43,001	10.90	29,744	6.67	—	—

In this table a direct comparison, which should only be made within one test, shows that insecticidal efficiency increases with viscosity. Similar results were obtained by these workers in insectary tests against codling moth eggs, as shown in Table VIII.

Table VIII.
Influence of viscosity on ovicidal value of oil
(from Spuler, Overley and Green (64)).

Spray strength %	Viscosity 50-55 sec. (Saybolt at 100° F.)		Viscosity 70-75 sec. (Saybolt at 100° F.)		Viscosity 110-120 sec. (Saybolt at 100° F.)	
	Total eggs	Percentage hatched	Total eggs	Percentage hatched	Total eggs	Percentage hatched
$\frac{1}{2}$	204	25	469	21	384	10
$\frac{3}{4}$	761	22	569	15	461	6
1	500	16	429	11	231	5
1 $\frac{1}{2}$	106	15	—	—	157	4

Against red spider species, Spuler, Overley and Green (63) reported that apple trees sprayed with oils of viscosities 50-55, 70-75 and 110-120 sec. Saybolt at 100° F. were free from these pests, but they stated that the oil concentration of the spray can be reduced if oils of viscosity 85-100 sec. are used instead of oils of 70-75 sec. viscosity. It is not clear from their report whether this reduction is to be associated with a greater acaricidal potency of the more viscous oils or with spray damage factors (see below). Penny (50), in discussing the general results of oil sprays on citrus red spider, stated that oils of viscosity greater than 65 sec. Saybolt at 100° F. are necessary to kill the eggs and that oils of 55 sec. viscosity failed to give more than a temporary control.

The conclusion is, therefore, that for the oil to be effective it should have a viscosity above a certain minimum and that there is evidence, in some cases, of a tendency for efficiency to increase with viscosity.

(b) *Chemical properties.*

It has been suggested by de Ong (10) that refinement may reduce insecticidal efficiency, as the more reactive hydrocarbons, which are presumably of greater toxicity than the bland saturated oils, are removed by the refining process. No evidence in support of this hypothesis can be obtained from de Ong, Knight and Chamberlin's work summarised in Table IV.

The results of Griffin, Richardson and Burdette given in Table VI indicate that toxicity to aphides is independent of base of oil and percentage unsulphonated residue, whilst English (15) found in laboratory tests, using San José scale, oyster-shell scale (*Lepidosaphes ulmi*) and

various species of aphides, that, in quick-breaking emulsions, less highly refined oils may be more effective than highly refined oils of equal viscosity, but came to the conclusion that a saturated oil may be more effective than an unsaturated oil because of its influence in some cases on the stability of the emulsion. Spuler, Overley and Green⁽⁶⁴⁾ reported that in insectary tests upon codling moth eggs the same rate of killing was obtained from oils of the same viscosity but varying 48 per cent. in unsulphonated residue. Ebeling⁽¹⁴⁾, discussing toxicity to citrus red scale, concluded that "in general the qualities of the spray making for greater safety (to the tree) also reduce insecticidal efficiency with the exception of sulphonation. An oil of highest sulphonation may be used without appreciable reduction in degree of control." In trials he found no correlation between degree of refinement and insecticidal efficiency.

It would appear, therefore, that the expected loss of insecticidal properties with refinement has not been established. The actual range of percentage unsulphonated residue to be adopted is determined more by the toxicity of the oil to foliage than by its insecticidal properties.

(c) *Oil concentration.*

Smith⁽⁶¹⁾ obtained evidence, from field trials with petroleum sprays on citrus, that the degree of control obtained with any one oil is directly proportional to the percentage of oil in the spray. From laboratory and field trials against camphor scale (*Pseudoanidia duplex*), Cressman and Dawsey⁽⁵⁾ similarly concluded that insecticidal efficiency varied directly with the oil concentration.

(d) *Type of emulsification.*

It would appear, from the American literature, that the first petroleum sprays used were made by vigorous agitation of oil and water with or without milk. They were therefore of an unstable quick-breaking type, and were replaced later by more stable soap emulsions and commercial preparations. The quick-breaking type again came into favour on citrus after de Ong and Knight⁽¹²⁾ found that from four to six times the amount of oil was required in a soap emulsion to yield the results obtained with either a lime or a calcium caseinate emulsion. Controlled work was carried out by de Ong, Knight and Chamberlin⁽¹³⁾. Using a highly refined lubricating oil at 2 per cent. and calcium caseinate at various concentrations as emulsifier, they found that approximately 40 per cent. of the scale (*Chrysomphalus aurantii*) survived when the emulsifier concentration exceeded 0.5 per cent., whereas all were killed at emulsifier concentrations below 0.125 per cent. This increased efficiency was found to run

parallel, firstly, to the amount of oil deposited upon a standard surface which was greater with an unstable emulsion than with a stable emulsion of the same oil content and, secondly, to the average size of the oil droplets in the emulsion which was greater the less stable the emulsion. Griffin, Richardson and Burdette⁽²¹⁾ obtained similar results, summarised in Table VI, in their study of the action of petroleum oil sprays on *Aphis rumicis*. The "hot 40 lb. pressure" method of emulsification with potash fish-oil soap yielded emulsions of which the average diameter of the oil droplets was 8-10 microns, whereas the cold-stirred method with cresol added to aid emulsification gave emulsions of which the average oil droplet diameter was only 2 microns. The lower insecticidal efficiency, to which the added cresol did not directly contribute, of the emulsions of smaller droplet size is clearly shown in Table VI. These investigators also correlated this reduced efficiency with the smaller amount of oil retained by the foliage with the emulsions of smaller droplet diameter.

English⁽¹⁵⁾, from laboratory studies using various species of aphides, concluded that the most effective emulsion was that which is relatively unstable but with high wetting ability, and that a relatively "poor-wetting" unstable emulsion may be more effective than a "good-wetting" stable emulsion. The relationship between concentration of emulsifier and insecticidal efficiency demonstrated by de Ong, Knight and Chamberlin has been tested by Smith⁽⁶¹⁾ and Ebeling⁽¹⁴⁾, who used the citrus red scale as the test organism and blood albumin as emulsifier. Similarly Cressman and Dawsey⁽⁵⁾ found that the insecticidal efficiency of oil-sodium oleate emulsions varied inversely with the concentration of soap in the aqueous phase of the emulsions. The decreased efficiency of the sprays when the amount of emulsifier used was increased was associated by these workers with the smaller amount of oil deposited on the sprayed surface.

It may therefore be concluded that the type and possibly the amount of emulsifier present in prepared products will have to be an item for control in the specification.

(ii) *Phytocidal action.*

In discussing the effect upon the plant of the summer application of petroleum oils it is convenient to distinguish two main types of injury: (a) chronic injury, which becomes apparent slowly and which, associated with metabolic disturbances, comprises such effects as the yellowing and early shedding of leaves and a delayed ripening and premature drop of fruit; (b) acute injury, in which leaf tissue may be killed within two or

three days of the spray application and which, being due to the direct action of phytocidal constituents, is determined more by the chemical than by the physical characteristics of the oil.

(a) *Physical properties.*

With those lubricating oils not causing actual burning and spotting of citrus foliage, de Ong, Knight and Chamberlin⁽¹³⁾ found that oils of low viscosity are apparently safer to use than those of high viscosity. They attributed this greater safety of the oils of low viscosity to their more rapid disappearance from the foliage, which they considered due primarily to absorption rather than to simple volatility. Knight, Chamberlin and Samuels⁽³³⁾ observed the penetration of the oil into the vascular system and showed that the recovery of citrus from the effects of oil sprays was more rapid, the lighter the oil applied. They considered the metabolic disturbances to be due to physical rather than chemical reactions, and concluded that, on citrus, oils of viscosity greater than 60 sec. Saybolt at 100° F. should be used sparingly.

On deciduous trees, Kelley⁽³¹⁾, who investigated the effect of hydrocarbon oils on transpiration rate, found that reduction of this rate was apparently due to the physical and not the chemical properties of the oil and concluded that high viscosity is associated with greater interference with metabolic processes. Spuler, Overley and Green⁽⁶⁴⁾, who employed the starch content of apple foliage as a measure of the effects of oil sprays on the metabolic processes, also found that the accumulation of starch in sprayed foliage increased with the viscosity of the oil applied. Further they reported that on trees receiving six applications of medium viscosity (70–75 sec.) or of heavy (110–120 sec. Saybolt at 100° F.) oil the fruit was smaller than on the trees not sprayed with oil washes, whereas the fruit on trees receiving a light (50–55 sec. Saybolt at 100° F.) oil was larger than on the control trees, the latter difference being explained by the presence of red spider on the control trees.

It may therefore be concluded that the higher the viscosity of the oil, and consequently the higher its boiling range, the more liable it is to produce injury of the chronic type.

(b) *Chemical properties.*

Gray and de Ong⁽¹⁹⁾, from the results of field trials on citrus, concluded that the phytocidal properties of oils were closely correlated with their content of the more reactive constituents which are removed by sulphuric acid treatment. On the basis of their work and the further

experiments of de Ong, Knight and Chamberlin (13), all but highly refined lubricating oils were eliminated for use on citrus.

To deciduous foliage, Spuler, Overley and Green (64) reported that highly refined oils are less toxic than those less refined. They found, by field trials on apple, with oils of 50 and 70 sec. Saybolt at 100° F., of which half of each sample had been refined by the sulphuric acid process and the other half by the liquid sulphur dioxide method, that no foliage damage resulted after three applications. It may therefore be concluded that the phytocidal constituents are removed by either refinement process.

Critical studies upon the constituents of petroleum oils responsible for acute phytocidal properties were made by Green (20a) and by Young and Morris (74). In both investigations confirmation was obtained of Gray and de Ong's observation that oil injury is correlated with the percentage of oil removed by sulphonation, though, in both cases, certain oils behaved exceptionally in that they produced greater injury than was accounted for by their content of reactive hydrocarbons. Tutin (69) used iodine value as a measure of the degree of refinement and concluded that this figure may be taken as an index of toxicity of a petroleum oil towards vegetation.

With regard to the phytocidal properties of the non-hydrocarbon constituents, such as the sulphur compounds suggested by de Ong (9) as probable causes of plant injury, Green was unable to obtain conclusive evidence. Whereas the content of nitrogenous derivatives gave no indication of the phytocidal properties of the oils tested, sulphur content showed some relationship. By adding to a saturated oil sulphur compounds of a type likely to be present in phytocidal petroleum oils, Green produced greater injury, but its extent was unrelated to the amount of the sulphur derivatives added.

(c) *Oil concentration.*

It appears to have been tacitly assumed that the extent of injury to foliage is proportional to the oil content of sprays prepared by any given emulsifier, and no evidence has been obtained which suggests the incorrectness of this assumption.

(d) *Type of emulsification.*

Spuler, Overley and Green (63) found that, on apple, there was a stage in the development, from the time when the buds showed green until the cluster of individual buds separated, when the application of quick-breaking ammonia-casein emulsions caused greater injury than the stable

miscible oil sprays. These workers suggested that quick-breaking emulsions should not be used after the dormant period, but it would appear that their results were obtained with oils of low unsulphonated residue. With summer oils they concluded that the type of emulsification is relatively unimportant from the standpoint of either insect control or plant injury.

There is, however, evidence that, even with highly refined oils, the quick-breaking emulsion is liable to cause greater injury than the stable emulsion, other factors being equal. Thus Young and Morris (74) preferred cresol-soap emulsions to caseinate emulsions, not only because they were more easily and uniformly applied, but because they were less injurious to apple leaves. Ebeling (14) concluded that the liability to injury is inversely proportional to the concentration of emulsifier used.

It would appear that the influence of stability of emulsion upon the phytocidal properties of the spray is related to the factor of oil deposition in that, the less stable the emulsion, the greater the amount of oil retained on the sprayed surface and the liability to cause injury.

(iii) *Specifications suggested elsewhere.*

From the results of the work outlined above and from general field experience of the use of petroleum oil emulsions on deciduous trees and on citrus, specifications have from time to time been suggested elsewhere. To enable a comparison of these specifications they are tabulated under physical and chemical criteria in Tables IX and X.

(iv) *Conclusions.*

Reviewing in turn the various physical and chemical criteria and their influence upon the insecticidal and phytocidal properties of the oil, it is possible to derive the following requirements for the specification of oils for summer washes.

(a) *Boiling range.* (b) *Volatility.* For reasons already given, boiling range is preferred to a volatility figure. Further, on the basis of critical work and general experience, boiling range is displacing volatility and viscosity as the physical characteristic in the classification of petroleum oils for use in the Californian citrus industry (see *e.g.* Smith (61)). The Bureau of Pest Control of the Californian Fruit Growers' Exchange (Woglum (71, 72)) has adopted boiling range, for "it would appear that this distillation test (percentage of oil distilled at various temperatures) arranges the oils in a general way very comparable to their scale-killing value and effect on trees under our conditions".

Table IX.
Physical criteria (summer oils).

Authority	Locality	Boiling range	Viscosity	Sp. gr. 60° F. 0-90; but cer- tain grades of 0-86 suit- able	Purpose
Chesnut Circ. No. 5 (1928)	British Isles	—	—	—	Red spider (glasshouse)
de Ong (11)	U.S.A.	—	70-100 sec. Saybolt at 100° F. 50-75 sec. Saybolt at 100° F. 95-110 sec. Saybolt at 100° F. 55-75 sec. Saybolt at 100° F.	— — — —	Red spider General Resistant insects (apple) General (apple)
Robinson, Fisher and Spuler (58)	Washington	—	—	—	—
Woodworth (73)	California	Below 325° C. Below 350° C. 10 % 30 % 20 % 50 % 25 % 75 %	110 sec. Saybolt at 100° F. 80 sec. Saybolt at 100° F. 60 sec. Saybolt at 100° F. Little less than 90 sec. Saybolt at 100° F.	0-885 (20° C.) 0-880 (20° C.) 0-874 (20° C.) —	Citrus: Thick grade (average) Medium grade (average) Thin grade (average) General (deciduous)
Quaintance, Newcomer and Porter (53)	U.S.A.	—	—	—	—
Newcomer and Yothers (47)	U.S.A.	—	65-75 sec. Saybolt at 100° F.	—	Codling moth (apple)
Smith (61)	California	At 575° F. At 700° F. <20 % >95 % (i) <15 % >90 % (ii) <10 % >85 % (iii) < 5 % >80 % (iv) 0 % >70 % (v)	— — — — —	— — — — —	General (citrus) Grade I General (citrus) Grade II General (oranges) (coastal districts) Grade III General (oranges) Grade IV Red scale (lemons) Grade V
Penny (50)	California	—	<65 sec. Saybolt at 100° F.	—	Red spider (citrus)
Cunningham and Muggeridge (7)	New Zealand	—	115-185 sec. Redwood 1 at 100° F.	—	General
Knight and Cleveland (34)	California	—	50-110 sec. Saybolt at 100° F.	—	General

Table X.
Chemical criteria (summer oils).

Authority	Locality	Percentage unsulphonated residue Free from unsaturated hydrocarbons	Iodine value	Emulsifier Ammonia casein (as small a quantity as possible)	Oil content 80 %	Purpose Red spider (glasshouse)
Cheshunt Circ. No. 5 (1928)	British Isles		—			
de Ong (10)	California	88-89 %; lower percentage only with oils of 40-50 sec. Saybolt at 100° F. ≤ 85 %	—	—	—	Citrus
Robinson, Fisher and Spuler (58) Woodworth (73)	Washington California		—	—	—	Apple
Newcomer and Yothers (47) Smith (61)	U.S.A. California	Thick grade 97 % (average) Medium grade 95 % (average) Thin grade 93 % (average) Large proportion of unsulphonated residue Grade I 90 % Grade II 91 % Grade III 92 % Grade IV 93 % Grade V 95 %	—	—	—	Citrus Apple
Tutin (69) Cunningham and Muggeridge (7) Young and Morris (74)	British Isles New Zealand Montana	— — ≤ 85; oils of only 90-91 % preferable	± 1 (for use at 3 %) ± 6 (for use at 1.5 %)	— Permanent type preferable	— — —	Deciduous Deciduous Apple

For the purposes of the citrus grower it has been found necessary (Woglum (72), Smith (61)) to specify five grades of oil, and it will be seen from Table XI that, although these two authorities employ different methods of expressing boiling range, they are in good agreement in the interpretation of the biological value of the various grades. It is for this reason, more than for the purpose of using their conclusions for the derivation of specifications for oils suitable for application to deciduous foliage, that the citrus work has been mentioned above. The need for the adoption of various grades for use on citrus in California seems to arise through the different relative susceptibilities of the resistant red scale and the easily killed black and *Citricola* scales, and through the different relative susceptibilities to damage of the orange and the less sensitive lemon tree.

Table XI.

Woglum (72)			Smith (61)				
Grade	% distil <335.5° C.	Purpose	Grade	Max. % distil at 302° C.	Min. % distil at 371° C.	% distil* <335.5° C.	Purpose
Light	65-100	Too light for general scale control	I	20	95	60-70	Off season for red spider and scale
Light medium	50-64	Black and <i>Citricola</i> scale	II	15	90	50-60	Black scale and red spider
Medium	40-49	Purple scale on oranges in coastal area	III	10	85	40-50	Scales and red spider on oranges in coastal districts
Heavy medium	30-39	Too heavy for use on orange	IV	5	80	34-45	Better than III, but heaviest oil which may be applied to orange
Heavy	0-29	For lemons only	V	0	70	30-35	Red scale on lemons only

* Interpolated from boiling-range curves.

There is, as yet, no evidence that, under our conditions, there is need to define different grades of summer oil for specific purposes, though it is possible that it may be found that the oils most suitable under glass-house conditions may not be those most suitable under orchard conditions. In the absence of data upon this point, it is suggested that as wide a boiling range as possible be chosen of oils which will, on the basis of the results given above, be efficient insecticides.

The results of critical and general work on the action of oils in controlling the pests of deciduous trees and herbaceous plants indicate that an effective oil will have a boiling range between the following limits:

Not less than 90 per cent. of the oil should distil above 300° C. and not less than 10 per cent. of the oil should distil below 330° C.

Not less than 50 per cent. of the oil should distil above 340° C. and below 365° C.

Not less than 20 per cent. of the oil should distil above 370° C. and not less than 80 per cent. below 390° C., all percentages being by volume.

The minimum boiling range, *i.e.* $\nless 90$ per cent. above 300° C., 50 per cent. above 340° C., and 20 per cent. above 370° C., is determined from the insecticidal aspect, whereas the maximum boiling range, *i.e.* $\nless 10$ per cent. below 330° C., 50 per cent. below 365° C. and 80 per cent. below 390° C., is determined from the phytocidal standpoint.

(*e*) *Flash and fire points.* May be neglected.

(*d*) *Viscosity.* Although for use on citrus it has been found necessary to specify five grades of oil, for reasons given under (*a*), one range will, on the present evidence, suffice for conditions in the British Isles. The minimum viscosity is determined by the minimum boiling range and, for highly refined oils, will approximate to 75 sec. Redwood 1 at 70° F. This figure, on the basis of the boiling-range data given by Marshall (36), corresponds roughly to 60 sec. Saybolt at 100° F., a figure which agrees with the minima suggested by American workers for use on deciduous trees. The maximum viscosity, in accordance with the boiling range chosen, will be 150 sec. Redwood 1 at 70° F., a figure which approximates to the maximum of 110–120 sec. Saybolt at 100° F. indicated by American investigators.

(*e*) *Pour and cold tests.* May be neglected.

(*f*) *Specific gravity.* The limits are fixed by the suggested boiling and viscosity ranges at 0.86–0.92 (60° F.).

(*g*) *Base of oil.* (*h*) *Colour.* May be neglected.

(*i*) *Unsulphonated residue.* General experience under English conditions has been obtained, up to the present, with oils of unsulphonated residue greater than 90 per cent. by volume, and it is suggested that this minimum be accepted in place of the minimum of 85 per cent. which is indicated by the results of American work on deciduous trees. The use of a higher figure in the case of the more viscous oils, as is the practice in the Californian citrus industry, does not at present appear necessary.

(*j*) *Iodine value.* (*k*) *Heat of bromination.* (*l*) *Solubility in dimethyl sulphate.* (*m*) *Sludge test.* May be neglected if (*i*) be given.

(*n*) *Sulphur content.* There is no experimental evidence that this item, suggested by de Ong (9) on the basis of the probable phytocidal properties of sulphur derivatives present in petroleum oils, is necessary. On the contrary, certain types of sulphur derivatives of petroleum hydrocarbons have been recommended as ingredients of foliage sprays. de Ong (11)

reported that certain cyclic sulphur derivatives are effective fungicides, and Martin (39) found that the alkali salts of beta-petroleum sulphonic acids are safe spray spreaders. A figure for sulphur content may therefore be misleading in determining the potential foliage-damaging properties of an oil.

A petroleum oil conforming to the specification deduced above is referred to, for convenience, as a Grade G oil.

In the case of manufactured preparations, oil content and types of emulsification have now to be considered.

(o) *Oil content.* As already pointed out, the amount of oil which can conveniently be incorporated in an oil preparation varies according to the type of emulsification used.

(p) *Types of emulsification.* It is the unanimous conclusion of critical and general work that the stability of an emulsion, as affected by type and concentration of emulsifier, has an important influence on its insecticidal properties, but it is less certain, though probable, that the unstable emulsion is more liable to cause foliage damage than a stable emulsion, other conditions being similar. With commercial preparations, however, a limit to instability is imposed by the manufacturer's requirement that this preparation will be safe in inexperienced hands and will dilute to give an emulsion of stability such that a uniform oil concentration is applied with the spray machinery normally used by the grower. There is, therefore, a tendency for the marketing of preparations which give stable non-creaming emulsions, a tendency which, in the case of summer washes, may be wrong. It is suggested that, provided the grower will co-operate by insisting on the thorough mixing of the contents of a drum of commercial preparations before withdrawing aliquots for dilution, the requirement be so worded that the creaming of a preparation of the stock emulsion type be permitted. This point is further discussed in connection with tar oil preparations (see p. 383).

The fact that the stability of the diluted emulsion after passage through the nozzle will be dependent on the spray machinery and on the purity of the water employed for dilution renders a rigid control of the stability of the emulsion impossible and a detailed specification regarding content and nature of emulsifier impracticable.

There is, however, an increasing use and scope for summer-oil preparations which can be used in combined washes with other insecticidal or fungicidal materials. Such combined washes not only effect great economy in time and labour but, in certain cases, the presence of the oil may increase the efficiency of its companion insecticide or fungicide,

e.g. by improving the adherence of the spray deposit as with lead arsenate (Spuler, Overley and Green (64)) or Bordeaux mixture (Martin (38)). It is obvious that, for use in these combined washes, the oil preparation must not contain, as emulsifier, constituents which react, with detrimental consequences, with the companion insecticide or fungicide. For example, the use with lead arsenate of an oil preparation containing free alkali or alkali salts yielding free alkali by hydrolysis, such as soaps or ammonia casein, may result in the production of soluble arsenic derivatives (Robinson (57)), which are known to be strongly phytocidal. Further, emulsifiers such as soaps, resins, and beta-petroleum sulphonates, which yield insoluble calcium salts, will interact with calcium derivatives such as lime sulphur, and the emulsion may break or invert with consequent application of a spray of non-uniform oil concentration.

It is, accordingly, necessary that the manufacturer should declare whether or not the preparation is suitable for use in combined washes containing lead arsenate, lime sulphur or Bordeaux mixture. This requirement need apply only to preparations of the stock emulsion type, for all miscible oil preparations are unsuitable for use in such combined washes because of the presence of alkali salts yielding soluble arsenic with lead arsenate or insoluble calcium salts with lime sulphur.

On this basis, it is suggested that commercial summer oil preparations should conform to the following requirements regarding oil content and type of emulsifier:

(1) Miscible oil type (M.O.): not less than 80 per cent. by weight neutral hydrocarbons.

(2) Stock emulsion type (S.E.):

Type I. Not less than 80 per cent. by weight neutral hydrocarbons.

Type II*a*. Not less than 66·7 per cent. by weight neutral hydrocarbons, containing constituents which render it unsuitable for use with lead arsenate, lime sulphur or Bordeaux mixture.

Type II*b*. Not less than 66·7 per cent. by weight neutral hydrocarbons, compatible with lead arsenate, lime sulphur or Bordeaux mixture;

the oil content being determined in all cases by the prescribed analytical methods.

The specification of (*a*) and (*b*) types, differing in suitability for use with lead arsenate, etc., for Stock Emulsion Type I preparations, is not at present necessary, for no example has yet been met with of a stock emulsion

preparation of not less than 80 per cent. neutral oils which can be recommended for use with lead arsenate, lime sulphur or Bordeaux mixture.

Intermediate bud-burst washes.

Consideration of the requirements of oils suitable for dormant and for summer use has led to the derivation of two specifications, one covering highly refined oils and one including the semi-refined lubricating oils. It has also been indicated that oils suitable for use during the intermediate period between the dormant and blossoming stages required separate mention. There are particular crops to which the semi-refined oils may be applied at bud burst without causing injury, whereas other plants may suffer damage by an oil not highly refined. Further, with any one crop, there may be a period during the development when an oil of relatively low degree of refinement may be applied with safety. Spuler, Overley and Green⁽⁶³⁾ found, for example, that, on apples, no injury was caused by the application of quick-breaking semi-refined oil emulsions up to the time the buds first showed green, nor for a short period after the cluster buds had separated, but that, in the intermediate period, injury was produced.

General experience and, to some extent, the critical work already described, shows that an oil of an intermediate grade of refinement may be suitable for application to plants to which the semi-refined oil is phytocidal.

It is suggested that a suitable intermediate oil would conform to the following specification (Grade F):

(a) *Boiling range.* Not less than 90 per cent. by volume to distil above 310° C., 50 per cent. above 345° C. and 80 per cent. above 375° C.

(d) *Viscosity.* Not less than 100 sec. and not greater than 400 sec. Redwood 1 at 70° F.

(f) *Specific gravity.* 0.86–0.92 at 60° F.

(i) *Unsulphonated residue.* Not less than 80 per cent. by volume.

The transitional stage between the dormant and blossoming periods is one in which combined insecticide fungicides are of great usefulness, and, with preparations intended for application during this period, it is necessary that the type of emulsifier present should be stated. For this purpose the method suggested for the summer petroleum washes is adopted.

(1) Miscible oil (M.O.): not less than 80 per cent. by weight neutral hydrocarbons.

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(2) Stock emulsion (S.E.):

Type I. Not less than 80 per cent. by weight neutral hydrocarbons.

Type II*a*. Not less than 66·7 per cent. by weight neutral hydrocarbons containing constituents which render the preparation unsuitable for use with lead arsenate, lime sulphur or Bordeaux mixture.

Type II*b*. Not less than 66·7 per cent. by weight neutral hydrocarbons, compatible with lead arsenate, lime sulphur and Bordeaux mixture.

Further, as the semi-refined petroleum oils covered by the winter oil specification (see p. 356) may be suitable in some cases for application as intermediate washes, it is suggested that the above three grades of stock emulsions be also adopted for such preparations. It is for this reason that the suggestions detailed in the schedule (see p. 395) for preparations containing the semi-refined petroleum oils differ from the suggestions derived from the consideration of general experience and critical experiment of dormant petroleum washes.

TAR OILS.

DEFINITION BY SPECIFICATION.

Tar oils are products of the destructive distillation of carbonaceous matter, the predominant sources being the high-temperature carbonisation of coal and the coke-oven processes. Like the petroleum oils, tar oils consist of complex mixtures mainly of hydrocarbons of which a complete analysis indicating the content of individual compounds is impossible. Other methods of definition are therefore employed and, for industrial purposes, the tars are classified according to the process from which they are derived, and the oils, obtained from the tars by distillation, are further classified, according to the boiling range of the fraction, into groups of which the creosote and anthracene oils have been utilised for insecticidal purposes. The term creosote oil, originally applied to certain wood tar oils, is nowadays used for any tar distillate of specific gravity greater than that of water, and comprises the distillate coming over between about 180° C. and the pitching point (360–400° C., according to the type of pitch required). The anthracene oils are those fractions from which crude anthracene separates on cooling and have a boiling range of about 270° C. to the pitching point.

The conception of a tar oil as being composed of compounds which fall into certain homologous series is of great assistance in defining tar oils more closely than by source and boiling range. The four groups of homologous series of hydrocarbons which constitute the petroleum oils, namely, paraffins, naphthenes, unsaturated hydrocarbons and aromatics, again occur, but the chief chemical distinction between tar and petroleum oils lies in the different proportions of the four groups present. The paraffins and naphthenes appear in small quantity in low-boiling tar oil, but are absent or unimportant in high-boiling tar oils, particularly from the coke-oven process or from the high-temperature carbonisation of coal. With such tar oils the predominant constituents are hydrocarbons of the aromatic series.

In addition to the hydrocarbons, creosote and anthracene oils contain hydrocarbon derivatives which also fall into homologous series and which possess acidic or basic properties which permit of their separation. One group is the tar acids which are mainly hydroxyl derivatives of aromatic hydrocarbons and are phenolic in character. The content of tar acids varies, in general, according to the source of the tar and the boiling range of the oil. Thus tar oils from the low-temperature carbonisation of coal contain greater quantities than those from high-temperature carbonisation, whilst during distillation a greater proportion of tar acids passes to the middle fractions (Morgan⁽⁴⁶⁾; Kester and Pohle⁽³²⁾). A second group, the tar bases, are soluble in acid and consist mainly of nitrogenous derivatives of the aromatic hydrocarbons, *e.g.* quinoline.

For industrial purposes the criteria employed to define a creosote or anthracene oil are limited to those mentioned above, namely, the physical characteristics of specific gravity and boiling range, and the chemical criteria of content of neutral hydrocarbons, tar acids and tar bases. As the neutral oils constitute so large a part of the tar oil and are separated as such in the analytical treatment of tar-oil preparations, it is more convenient to consider the physical characteristics of the neutral hydrocarbons only and not those of the total tar oil. It is upon the relationship of these items to insecticidal properties that critical work has been carried out with the tar oils, from which specifications of oils suitable for use in sprays may be deduced. In addition to these items, the question of the presence of solid anthracenoid hydrocarbons, and, with tar oil preparations, the influence of the type of emulsification and emulsifier must be considered.

Tar oil preparations suitable for the preparation of sprays by the grower by direct dilution with water are, as with the petroleum oil pre-

parations, of two types, the miscible oils and stock emulsions. A third type, the two-solution wash, which requires addition to alkali for the emulsification of the oil, has been introduced as a manufactured wash and the question of the need for such a type must be examined.

BIOLOGICAL PERFORMANCE AND CHARACTERISTICS.

Tar oils, because of their phytocidal properties, are used only as winter washes for application to dormant trees, when they function mainly as ovicides, and for the destruction of moss and lichen. As critical work upon insecticidal properties has been carried out with fractions separated by the analysis of tar oils and tar oil preparations, and because the neutral oils constitute by far the greater proportion of the liquid oil, this work has been concerned mainly, on the physical side, with the neutral oils only.

(i) *Insecticidal action.*

(a) *Physical properties (of neutral oils).*

For the examination of the ovicidal constituents of a successful winter-wash preparation, Tutin (68) fractionated the neutral hydrocarbons into high- and low-boiling material and, in preliminary trials on eggs of *Aphis pomi*, found the high-boiling fraction of greater toxicity. In later trials, with eggs of *Cheimatobia brumata*, he separated the neutral oils from a vertical retort tar into high-boiling (280–360° C.) and low-boiling (190–280° C.) fractions and the tar acids into high-boiling (280–360° C.) and low-boiling (190–220° C.) fractions. The percentage hatches of eggs sprayed with emulsions of these products are given in Table XII and indicate that the high-boiling fraction is the more effective ovicide.

Table XII.

Concentration of oil (%)	Percentage hatch	
	5	8
Total tar oil	2.9	1.5
Low neutral	3.2	0.4
High neutral	0.0	0.0
Low neutral + 20 % low tar acids	13.9	1.9
High neutral + 20 % high tar acids	0.3	0.0

Goetze (16), who used the grain weevil (*Calandra granaria*) as test organism, also showed that insecticidal activity increased with the boiling range of both total and neutral oils isolated from representative tar oil preparations. He concluded that these products, independently of the action of the tar acids and tar bases present, possess a greater insecticidal value the larger the fraction of boiling-point greater than 260° C. A

similar conclusion was derived by Beran and Watzl⁽⁴⁾ from a study of the action of tar oil fractions upon both *C. granaria* and larvae of the scale insect, *Lecanium corni*. These investigators showed the increase of toxicity of the lower hydrocarbons with boiling-point and the greater insecticidal action of heavy (14 per cent. boiling below 270° C.) over light (100 per cent. boiling below 270° C.) tar oils. Hartzell and Parrott⁽²⁵⁾ stated that certain results obtained in field trials with tar oil preparations could be explained on the basis that the higher boiling material is more toxic than the low-boiling fractions, but that tests on eggs of *Aphis pomi* indicated that even the fraction of boiling range 200–270° C. is very toxic.

Critical work would therefore indicate that the general insecticidal activity of tar oils increases with the boiling range of the neutral oils, a conclusion which is supported by data derived from petroleum oil trials and is a corollary of the hypothesis that insecticidal action is associated with the permanence of the oil film left after spraying. It will, however, be seen from the discussion of the relationships between insecticidal action and chemical properties that, in the case of tar oils, a second mode of action is possible which will have to be considered in drawing conclusions regarding the physical requirements of suitable tar oils.

(b) *Chemical properties.*

(i) *Source of tar.* In addition to the trials summarised in Table XII, in which a vertical retort tar was used, Tutin⁽⁶⁸⁾ carried out similar trials with fractions obtained from a horizontal retort tar and a coke-oven tar and obtained similar results. He concluded that the liquid neutral material of boiling range 280–360° C. appears to be essentially the same whatever tar it is derived from. This conclusion was confirmed by later work, recorded by Staniland, Tutin and Walton⁽⁶⁵⁾, in which the “high neutral” oils from a low-temperature carbonisation tar were shown to have an ovicidal action upon eggs of the small winter moth equal to the “high neutral” oil from a high-temperature carbonisation process. Hurt⁽³⁰⁾ also found that, although tar oils from different sources may differ in composition, yet tar oils of the same boiling range are, irrespective of their origin, about equally effective in their ability to destroy insect eggs. It may be concluded, therefore, that tar oils of similar physical and chemical characteristics have insecticidal properties independent of the source from which the tar was obtained.

(ii) *Neutral oils.* General experience and critical work has shown that suitable tar oils are effective against the eggs of Aphididae and

Psyllidae, against which petroleum oils are ineffective. It has been suggested by Staniland, Tutin and Walton⁽⁶⁵⁾ that this difference may be explained by the assumption that tar oils possess a definite toxic action, chemical in character, in addition to the physical "stifling" action to which the ovicidal action of tar and petroleum oils against geometrid and capsid eggs may be attributed. The chemical toxicity of tar oils was found by Austin, Jary and Martin^(2, 3) in field trials with petroleum oils of low refinement and tar oils to be associated with the content of aromatic hydrocarbons as determined by the percentage soluble in dimethyl sulphate. It is possible that this type of action is not so closely related to boiling range as is the "stifling" action which, as shown above, and in the case of petroleum oils, is dependent on the permanence of the oil film remaining after spraying. As already mentioned, Tutin recorded that the action of neutral tar oils on eggs of *Aphis pomi* increases with boiling range, whereas Hartzell and Parrott and general experience on the Continent (*e.g.* analyses given by Proffit⁽⁵²⁾ and by Beran⁽⁴⁾) indicate that tar oils of lower boiling range may be effective against aphid eggs.

With regard to the physical "stifling" action, there is evidence that the predominant groups of homologous series of hydrocarbons should preferably be insoluble in dimethyl sulphate. Austin, Jary and Martin^(1, 2) showed that against the eggs of the capsid *Lygus pabulinus*, the neutral hydrocarbons of tar oils are less effective than those of petroleum oil, and they showed⁽³⁾ that this lower toxicity is associated not only with the lower boiling range of the tar oils but with the type of hydrocarbon present.

(iii) *Tar acids.* Little work appears to have been carried out on the direct insecticidal action of tar acids *per se*, but Beran and Watzl⁽⁴⁾ found low-boiling phenols only moderately effective against *Calandra granaria* and *Lecanium corni*. Evidence upon the influence of the presence of tar acids on both the chemical toxicity and physical action of the neutral oils is conflicting. Tutin⁽⁶⁸⁾, from the results given in Table XII, claimed that the presence of acids lowers the toxicity of the tar oil towards the eggs of *Aphis pomi* and *Cheimatobia brumata*. Austin, Jary and Martin⁽³⁾ found that the removal of tar acids did increase the ovicidal efficiency of a tar oil against eggs of *Lygus pabulinus*, but in earlier trials⁽²⁾ obtained results with various tar oils which did not indicate any close correlation between toxicity and tar acid content. Massee, Steer and Goodwin⁽⁴²⁾ found that two tar oil preparations, each containing equal amounts of creosote oil emulsified similarly with a resin-caster oil soap but one containing 25 per cent. high-boiling phenols

(220–300° C.), the other, 25 per cent. low-boiling phenols (190–215° C.), had a similar ovicidal action against eggs of *Notolophus (Orgyia) antiquus*. Goetze⁽¹⁶⁾ found that the removal of tar acids and bases decreased the activity of tar oils against *Calandra granaria*, and that, in the case of the eight tar oil preparations examined, the tar acids played an important role though the toxicity of the preparations was mainly dependent on the neutral oils. Lindblöm and Sjöberg⁽³⁵⁾, on the other hand, concluded that a high content of tar acids does not appear to produce a greater toxicity of tar oil preparations to *Psylla mali*. It may therefore be concluded that, as the tar acids do not contribute as effectively as do the neutral oils to the insecticidal properties of tar oils, the presence of a relatively large proportion of tar acids is undesirable if it involves a reduction of the content of neutral oils.

(iv) *Tar bases*. The insecticidal activity of pyridine and quinoline, as typical tar bases, was examined by Beran and Watzl on *Calandra granaria* and *Lecanium corni*. The higher boiling quinoline was found to be the more effective. Tutin stated that such tar bases are known to be practically valueless for egg-killing purposes. On the part played by tar bases in tar oil preparations, Lindblöm and Sjöberg⁽³⁵⁾ concluded that their presence seemed to increase the efficiency of tar oil preparations against *Psylla mali*, but Goetze⁽¹⁶⁾ found the tar bases quite unimportant in determining the toxicity of tar oils to *Calandra granaria*. There is accordingly no consistent evidence that the tar bases play any important part in the ovicidal action of tar oils.

(v) *Solid hydrocarbons*. Both creosote and anthracene oils, on cooling, deposit solid hydrocarbons consisting mainly of naphthalene or anthracene according to the boiling range of the oil. The presence of such solid matter is objectionable from the practical aspect in that it may clog the spray appliances, but it has to be shown that these constituents do not contribute to the insecticidal efficiency of the oil before their removal can be recommended. Staniland, Tutin and Walton⁽⁶⁵⁾ showed anthracene to be ineffective against eggs of *Cheimatobia brumata*, Goetze⁽¹⁶⁾ found that naphthalene had no action on *Calandra granaria*, and Beran and Watzl⁽⁴⁾ proved naphthalene and anthracene to be of little activity, compared with high-boiling neutral oils, against *C. granaria* and *Lecanium corni*. Naphthalene and anthracenoid hydrocarbons separating from tar oils on cooling therefore appear to contribute little to the insecticidal efficiency of tar oils.

(vi) *Oil concentration*. Goodwin, Massee and Le Pelley⁽¹⁷⁾ attributed the exceptional toxicity of a wash containing 5 per cent. of tar oil pre-

paration to eggs of *Notolophus antiquus*, when compared with other dilutions, to a probable source of error, and, in a repeat experiment, Massee, Steer and Goodwin⁽⁴²⁾ showed that the toxicity rises normally with the concentration of the oil in the wash. Goetze⁽¹⁶⁾ obtained evidence, in his work on the toxicity of tar oil preparations to *Calandra granaria*, that the activity at different dilutions did not always run parallel to concentration. He did not consider the irregularities important, and most workers have accepted without question the conclusion that the insecticidal activity will increase normally with the oil concentration.

(vii) *Type of emulsifier*. Staniland, Tutin and Walton⁽⁶⁵⁾ reported that, in field trials, commercial preparations of high-boiling neutral tar oils were somewhat less effective than two-solution Agral W.B. emulsions, suggesting that this inferiority was associated with the difference in emulsification. On the other hand, Austin, Jary and Martin⁽²⁾ and Steer⁽⁶⁶⁾ obtained similar results with a tar-petroleum oil mixture whether emulsified by Bordeaux mixture or by the two-solution oleic acid method. Speyer⁽⁶²⁾ examined the importance of emulsion as a factor in determining the action of a number of manufactured preparations upon the eggs of *Psylla mali*. He found no relationship between toxicity and the stability or surface tension of the diluted washes.

It is possible that the differences found by Staniland, Tutin and Walton⁽⁶⁵⁾ are explainable in either of the following ways. Firstly, as these authors give particulars only of the dilutions of the concentrates used, the oil content of the wash prepared from the commercial preparations may have been, in general, lower than that of the Agral W.B. wash. Secondly, it is probable that the commercial concentrates used were of the stock emulsion type, and the spray-gang, if inexperienced in the application of washes prepared from the first commercial preparations of this type, may not have sprayed the trees so well as with the Agral W.B. wash. It was often advanced by growers, as an objection to concentrates of the stock emulsion type, that it is more difficult to distinguish the parts of the tree sprayed than in the case of the free-running clearly-visible resin-soap emulsion. With experience, however, this apparent disadvantage disappears, and it is for this reason, and because it is now generally realised by growers that correct spray application is essentially a skilled occupation, that this factor was not considered in the discussion of the relative merits of the miscible oil and stock emulsion types of preparation.

It may therefore be concluded that, as with the winter petroleum

washes, the method of emulsification is without effect upon ovicidal properties.

(ii) *Phytocidal action.*

Critical work with early tar oil washes by Molz⁽⁴⁵⁾ showed that inherent phytocidal properties rendered them quite impossible for use on foliage. This conclusion is in line with later work with petroleum oils which has shown that the foliage-injuring constituents are the unsaturated and aromatic hydrocarbons. The general association of foliage-injuring properties with aromatic character is shown by spray trials carried out by Martin and Salmon^(40, 41).

Investigations have, however, been carried out on the phytocidal properties of tar oils, in particular upon the buds of deciduous trees. Goetze⁽¹⁶⁾, in trials upon twigs of gooseberry, currant and privet, found that the increase of bud damage with the boiling range of the neutral tar oils was quite insignificant, and he concluded that the best insecticidal fractions were, at the correct concentrations, without phytocidal action if applied at the right time. Beran and Watzl⁽⁴⁾, who experimented on foliage buds of plum, found no simple relationship between the extent of bud damage and the content of high-boiling constituents of the tar oils applied.

Tutin⁽⁶⁸⁾ reported that the removal of tar acids not only increased ovicidal efficiency but reduced the phytocidal properties of high-boiling tar oils, the treated oils causing less injury to plum foliage and to young mustard plants. Goetze⁽¹⁶⁾, on the other hand, obtained no bud injury to gooseberry, currant or privet twigs sprayed with tar acids at 10 per cent., although injury followed the application of neutral oil at this concentration. Beran and Watzl were unable to demonstrate any simple relationship between damage to plum foliage buds and the tar acid content of the sprays.

That tar bases may be responsible for bud damage was suggested by Houben and Hilgendorff⁽²⁸⁾, who used quinoline (and also tar acids) at 50 per cent. concentration. Goetze⁽¹⁶⁾ found that, at the lower concentrations used in practice, these materials had a favourable influence on bud development.

There is, accordingly, no evidence that factors concerning the action of tar oils upon the dormant trees need be considered in deriving specifications of tar oils suitable for use.

The phytocidal properties of tar oils are, however, of practical use in the control of lichen and moss upon the sprayed trees. No critical

work appears to have been carried out upon the factors associated with lichen-killing properties with the exception of trials, recorded by Profft⁽⁵²⁾ and Goetze⁽¹⁶⁾, in which various carbolineum preparations failed, at 10 per cent., to kill certain lichens. General experience would suggest that, as the more recent manufactured products appear to be less effective in cleaning the tree of moss, etc., than the earlier types of tar oil wash, the phytocidal properties of the aromatic hydrocarbons and the tar acids play an important part, but this evidence is too indefinite to form the basis of definite recommendations.

(iii) *Specifications suggested elsewhere.*

The continental investigators have been concerned with specifications applicable to tar oil preparations rather than to the actual tar oils. It is, however, clear from their conclusions, summarised in Table XIII, that Houben⁽²⁷⁾, Profft⁽⁵²⁾, Goetze⁽¹⁶⁾ and Beran and Watzl⁽⁴⁾ are agreed that the boiling range of the oil should be as high as possible. This conclusion supports that of Tutin⁽⁶⁸⁾, who found that the neutral oil of boiling range 280–360° C. was the most effective. Theoretically, it is the corollary of the hypothesis that toxicity is due to a “stifling” action.

Table XIII.

Specifications suggested for tar oils and tar oil preparations.

		Physical criteria			Chemical criteria		
		Presence of solids	Stability of preparation	Boiling range of oils	Oil content	Tar acids	Tar bases
Tutin ⁽⁶⁸⁾	Oil	Nil	—	280–360° C.	—	≥ 2 %*	—
Houben ⁽²⁷⁾	Preparations	Nil	10–15 % emulsion to show no oil separation after standing 72 hrs.	≤ 20 % of oils to boil above 270° C.	≤ 60 %	≥ 15 %	≥ 4 %
Profft ⁽⁵²⁾ and Goetze ⁽¹⁶⁾	Preparations	As small as possible	Emulsified wash to be uniform and stable	≤ 200° C. preferably ≤ 250° C.	≤ 50 %	≥ 15 %	≥ 5 %
Hurt ⁽³⁰⁾	Oil	Nil after exposure at 40° F.	—	225–400° C.	—	Preferably removed	—
Beran and Watzl ⁽⁴⁾	Preparations	Nil	Concentration at which to be used to give stable emulsions with distilled water; 100ml. 10 % emulsion not to de-emulsify at once with 15 ml. N/10 MgCl ₂	≤ 40 % of oil to distil above 220° C. and ≤ 20 % above 270° C.	≤ 60 %	≥ 15 %	Unimportant
Hartzell and Pearce ⁽²⁶⁾	Oil (for aphid control only)	—	—	200–355° C., ≥ 15 % below 230° C.	—	≥ 5 %	—

* This limit was suggested to writer, in *litt.*, 4, x, 30.

On the other hand, Hurt⁽³⁰⁾ suggested that tar distillers should standardise tar oils for spray purposes at the boiling range of 225–400° C., whereas Hartzell and Parrott⁽²⁵⁾ obtained a similar degree of control of aphides, scale insects and eye-spotted bud moth with preparations containing tar oils of boiling range 200–360° C., 240–360° C. and 280–360° C. General experience on the Continent also indicates that, in so far as the chemical toxic action of tar oils upon eggs of aphides and psyllids is concerned, a tar oil of lower boiling range than that suggested by Tutin (*i.e.* 280–360° C.) may be effective. When the physical “stifling” action is required it may, however, be concluded that the tar oil should be of the highest practicable boiling range.

The requirements concerning the tar acid content differ greatly, but these differences may perhaps be explained in the following manner. There is experimental evidence (Tutin⁽⁶⁸⁾; Austin, Jary and Martin⁽³⁾) that the presence of excess tar acids may decrease the physical “stifling” efficiency of tar oils, and hence the recommendations for the small tar acid content. It is probable that this decrease of efficiency is not so marked in the case of the chemical toxic action, when the permanence of the oil film would not appear so important. Hence the relatively high maximum suggested by the Continental workers.

(iv) *Conclusions.*

On the basis of the critical and general work described above, it is suggested that tar oils fulfilling the following requirements are suitable for use in winter washes. In view of the two types of insecticidal action shown by tar oils, two grades are required, one, Grade A, which is for use in cases when the oil is expected to contribute to the control of insects of which the eggs are susceptible to the physical stifling action in addition to insect eggs sensitive to the chemical toxic action; the other, Grade B, where only the chemical toxicity to aphid and psyllid eggs is required. As the predominant insecticidal constituent has been shown to be the neutral oils this item is considered first.

(a) *Neutral oil content.*

The weight of neutral oils isolated from a Grade A tar oil should not be less than 88 per cent. This requirement covers the majority of strained anthracene oils of the boiling range suggested below. For Grade B tar oils in which, on account of the lower permissible boiling range, the content of tar acids in the oil may be greater, a permissible minimum of 75 per cent. by weight neutral oil is suggested.

(b) *Boiling range (of neutral oil).*

As tar oils of boiling range above 280–360° C. solidify on cooling, it is inadvisable to exceed this range. To allow for pyrolysis the following is suggested as suitable for tar oils intended for general winter wash purposes (Grade A): 90 per cent. by volume above 270° C., 50 per cent. above 325° C., and 20 per cent. above 365° C. If, however, the control required is limited to aphid and psyllid eggs, a lower boiling range may give good results. The analyses of typical present-day winter washes and Continental experience suggest that the following minimum range is suitable for Grade B oils: 90 per cent. by volume to distil above 230° C., 50 per cent. above 290° C., and 20 per cent. above 335° C.

(c) *Specific gravity.*

Because of its diagnostic value in distinguishing tar from petroleum oils it is suggested that the specific gravity be given in addition to solubility in dimethyl sulphate. Grade A tar oils should have a sp. gr. of 1.09–1.11 (60° F.), whilst Grade B should have a sp. gr. of 1.05–1.11 (60° F.).

(d) *Dimethyl sulphate solubility.*

The oil shall be completely soluble in dimethyl sulphate when tested by the prescribed method of analysis. This criterion, which gives information concerning the chemical toxicity of the oil, should be given for both Grade A and Grade B tar oils.

Concerning the constituents of tar oils other than the neutral oils:

(e) *Tar acids.*

Because the tar acid content decreases as the boiling range increases it would appear unnecessary to suggest a maximum permissible content if the required percentage of neutral oils is present. In the case of tar oil preparations, however, phenols are frequently added as they function as mutual solvents enabling or facilitating the solution of soaps in the tar oil (Smith⁽⁶⁰⁾), and with tar oil sprays intended for general use there is evidence that excess of tar acids is detrimental to insecticidal efficiency. It is suggested, therefore, that in the case of tar oil preparations for general use, the permissible tar acid content should not exceed 6 per cent. of the content of neutral tar oils. For preparations of Grade B tar oils, Continental experience leads to the suggestion that a maximum of 15 per cent. of the content of neutral oils is permissible.

(f) *Tar bases.*

With the exception of Lindblöm and Sjöberg⁽³⁵⁾, previous workers are agreed that the tar bases play no useful part in determining the biological performance of the oils, and it is therefore suggested that, provided the content of neutral oils be given, the content of tar bases may be neglected.

(g) *Content of solid matter.*

For phytocidal and practical reasons the presence of solid anthracenoid hydrocarbons is undesirable, and as these solids do not contribute to insecticidal efficiency it is suggested that the content of solid matter should never exceed 5 per cent. by weight of either tar oils or tar oil preparations. This requirement is fulfilled by most strained anthracene oils which have been refrigerated for the removal of anthracene.

In the case of tar oil preparations, it is necessary now to consider suitable specifications for tar oil content and for type of emulsifier. Considering first the latter item, for upon it depends the suitable minimum oil content, experimental evidence suggests that the type of emulsifier is unimportant from the biological standpoint. For convenience of handling, however, there are reasons for preferring the miscible oil type which, in general, is unsuitable for use with abnormally hard waters. It is therefore suggested that, as with the winter petroleum spray preparations, the manufacturer should specify whether the preparation is of the miscible oil (M.O.) or stock emulsion (S.E.) type. In the case of the petroleum oils it was found necessary, in order to cover existing preparations, to suggest two types of the stock emulsion preparation, but, as no tar oil preparation of the stock emulsion type has appeared with a content of 80 per cent. or more of tar oil, this type of high oil content is not at present required.

It will be seen from Table XIII that the Continental investigators have suggested conditions, to be fulfilled by tar oil preparations, concerning the stability of the emulsion. There is, however, no experimental evidence that the tests proposed will reflect the stability of the diluted emulsion as prepared in the field. Thus the behaviour of the preparation when diluted with distilled water may not resemble its behaviour when diluted with dyke water, for which reason Beran⁽⁴⁾ has suggested the use of a standard magnesium chloride solution for the stability of emulsion test. This test was apparently derived for use with tar oil preparations containing resin soaps, preparations which, in this country, would not be recommended for use with abnormally hard waters. The practical

value of such tests has been questioned by Speyer⁽⁶²⁾. For reasons already given for summer petroleum oils (p. 369) it is suggested that no specific recommendations be made on the basis of a stability of emulsion test.

On the experimental evidence, the neutral oils constitute the only important insecticidal constituents of tar oils, and it is suggested that the content of neutral oils is a more convenient particular than the actual tar oil content. This procedure, which has been adopted by most previous investigators, follows the proposed analytical technique and avoids confusion in cases where a separate statement of the content of tar acids is required.

The following requirements are accordingly suggested:

(1) Miscible oil type:

Grade A. Not less than 70 per cent. by weight of neutral oils;
not more than 4.2 per cent. by weight tar acids;

Grade B. Not less than 60 per cent. by weight of neutral oils;
not more than 9 per cent. by weight tar acids.

(2) Stock emulsion type:

Grade A. Not less than 60 per cent. by weight of neutral oils;
not more than 3.6 per cent. by weight tar acids;

Grade B. Not less than 50 per cent. by weight of neutral oils;
not more than 7.5 per cent. by weight tar acids;

as determined by the prescribed methods of analysis.

The neutral oils isolated from such preparations should conform to the specification given for Grade A and B tar oils respectively with one slight exception. It has become the practice to include in the so-called standard tar oil preparations a small proportion of high-boiling petroleum oils. The purpose of this addition is, according to Hough⁽²⁹⁾, to improve emulsification, but as the tendency is for the proportion of petroleum oils to be increased from approximately 10 per cent. to as high as 30 per cent., a possible explanation is that experience has shown that, at the dilution recommended, a reduction of tar oil content without loss of efficiency against aphid and psyllid eggs is possible. The reduced tar oil content enables the manufacturer to add high-boiling petroleum oils and so improve the general ovicidal properties of the preparation. The manufacturer has therefore modified the tar oil preparation to tar-petroleum oil preparations, for which specifications are suggested below. In view of the possible advantages of a small content of petroleum oils it is suggested that, for tar oil preparations, the characteristics required of

the neutral oil should be modified to include oils containing up to 10 per cent. of high-boiling petroleum oil. At least 90 per cent. of the neutral oil should therefore be soluble in dimethyl sulphate, and the specific gravity of the neutral oil may fall to the minimum of 1.06.

There is, as yet, no evidence to indicate that a separate grade to cover two-solution washes is necessary. The two-solution washes, in which a solution of fatty acids, sulphonated fatty oils or petroleum sulphonic acids in the oil is added to dilute alkali, are a convenient type of home-prepared wash, and, if the grower prefers to prepare his washes direct from tar and petroleum oils by such methods, he is covered by the specifications already suggested for these oils.

COMBINED TAR-PETROLEUM OIL PREPARATIONS.

It has already been shown that tar oils of suitable specification are toxic to the eggs of aphids and psyllids but are less effective than selected petroleum oils against the eggs of geometrids and capsids. Such petroleum oils, on the other hand, are ineffective against aphid and psyllid eggs, but, in addition to their action upon the eggs of geometrids and capsids, they are also effective to some extent against the eggs of acarids. It is, therefore, obviously advantageous, in cases when it is possible, to combine tar and petroleum oils in a dormant wash. The practice of mixing separate tar and petroleum oil preparations (see Hartzell and Pearce (26)) may be dangerous unless the emulsifiers present are known; and commercial preparations, containing mixtures of tar and petroleum oils as the active ingredients, have been introduced.

Of such combined preparations the critical work described above demonstrates two obvious requirements, firstly, that the content of tar oils shall be sufficient to ensure an aphid and psyllid control at the concentration advised by the manufacturer, secondly, that the tar oil shall be of the type which contributes most effectively to the insecticidal action of the petroleum oil. The first requirement is met by specifying the minimum content of neutral oils soluble in dimethyl sulphate. To fulfil the second requirement it is suggested that the tar oil present should conform to the Grade A specification given above.

The maximum concentration of the preparation which may be applied depends on its oil content and on the species and variety of plant sprayed. Experience has shown, for example, that black currants will tolerate a concentration of tar-petroleum oils that causes injury to apple. As the concentration of tar oil required for aphid control does not appear to

exceed 2.5–3.0 per cent. (Hurt⁽³⁰⁾, Hartzell and Pearce⁽²⁶⁾), it is suggested that two grades of tar-petroleum product will cover all practical requirements. For oil-tolerant crop plants a combination of tar-petroleum oils in the ratio 3 : 6, and for less tolerant species a ratio 3 : 3 is suggested as the basis for the derivation of suitable specifications. For convenience these preparations are called D and C grades respectively.

From these considerations the required specifications follow for the combined tar-petroleum oil preparations. As these materials are suitable only for dormant use there is no need to specify type of emulsifier beyond the miscible oil type based on a permissible minimum of 80 per cent. tar-petroleum oil and the stock emulsion type with the minimum of 66.7 per cent. tar-petroleum oil. Further, as the tar oil used is to conform to the Grade A specification, the neutral oil and tar acid content are based upon the permissible minimum and maximum respectively for this grade.

An adequate control of the characteristics of the neutral oil present is obtained by specifying the specific gravity and boiling range and the chemical items of solubility in dimethyl sulphate and of unsulphonated residue. The purpose of the dimethyl sulphate test is to ensure a sufficient yet not excessive content of the aromatic derivatives toxic to aphid and psyllid eggs. As the suggested analytical method gives the percentage of oil insoluble in dimethyl sulphate, and because this figure is on a different part of the scale to the unsulphonated residue figure, confusion is avoided by specifying the permissible maximum percentage of oil insoluble in dimethyl sulphate. Broadly speaking, the percentage insoluble in dimethyl sulphate gives, with the high-boiling oils under discussion, an approximately quantitative indication of the content of the saturated and unsaturated hydrocarbons which constitute petroleum oils.

It has already been shown (p. 349) that the ovicidal properties of petroleum oils used as winter washes may decrease when the degree of refinement is below that represented by a minimum percentage unsulphonated residue. This permissible minimum has therefore to be specified, a requirement which ensures a suitable content of saturated hydrocarbons. The combination of the two items of the permissible *minimum* percentage unsulphonated residue and the permissible *maximum* percentage insoluble in dimethyl sulphate should ensure, in conjunction with specific gravity and boiling range, that the oils present in combined tar-petroleum oil preparations will be of the proportions and characteristics required for winter wash purposes.

These suggestions may be summarised as follows:

Preparations containing Grade C oil.

(1) *Miscible oil type* (M.O.). Not less than 74 per cent. neutral oils; not more than 2 per cent. tar acids; not more than 5 per cent. solid matter;

(2) *Stock emulsion type* (S.E.). Not less than 62 per cent. neutral oils; not more than 1.5 per cent. tar acids;

as determined by the prescribed methods of analysis.

The neutral oil isolated from this type of preparation shall conform to the following requirements:

Boiling range. 90 per cent. to distil above 280° C., 50 per cent. above 335° C. and 20 per cent. above 370° C.

Specific gravity. Not less than 0.99 at 60° F.

Dimethyl sulphate, percentage insoluble in. Not more than 53 per cent. by volume.

Unsulphonated residue. Not less than 32 per cent by volume.

Preparations containing Grade D oil.

(1) *Miscible oil type* (M.O.). Not less than 75 per cent. neutral oils; not more than 2 per cent. tar acids and 5 per cent. solid matter;

(2) *Stock emulsion type* (S.E.). Not less than 63.5 per cent. neutral oils; not more than 1.5 per cent. tar acids;

as determined by the prescribed methods of analysis.

The neutral oils isolated from this type of preparation shall be of:

Boiling range. 90 per cent. to distil above 290° C., 50 per cent. above 340° C. and 20 per cent. above 375° C.

Specific gravity. Not less than 0.95 at 60° F.

Dimethyl sulphate, percentage insoluble in. Not greater than 70 per cent. by volume.

Unsulphonated residue. Not less than 42 per cent. by volume.

THE STATUS OF CERTAIN OILS AND PREPARATIONS UNDER
THE PROPOSED SPECIFICATIONS.

To illustrate the application of the suggested specifications, the results of the analysis of a number of oils and oil preparations are given in Tables XIV *et seq.* The samples analysed included typical products in general use. As others were submitted by biologists who, at the same time, carried out field trials with the washes and a number were submitted by manufacturers for an opinion of their probable worth as spray materials, all do not necessarily represent products placed on the market.

In many cases the results of replicate determinations are given in order to show the order of accuracy attainable under routine conditions with the proposed methods of analysis.

Tables XIV and XV contain analytical figures for samples of tar and petroleum oils respectively, and Tables XVI, XVII and XVIII figures for preparations (ready for use) containing tar oils, tar-petroleum oil mixtures and petroleum oils respectively.

Of the *tar oils* described in Table XIV, samples 2, 3, 11, 12, 13, 14, 15 satisfy the requirements suggested for Grade A oils, whilst samples 8 and 9 fail only because of a small content of low-boiling oils. All samples with the exception of No. 10 fulfil the requirements of Grade B oils. It may be noted that, in the case of sample 10, the tar distiller admitted that, by accident, the oil was unsatisfactory, and allowed the grower a rebate. With the exception of oils 8 and 9, which were not used, the oils proved satisfactory at 3 or 4 per cent. in field trials for the control of *Aphis* and *Psylla*. At 4 per cent. even sample 10 gave a satisfactory control of apple sucker (Austin, Jary and Martin (3)), an indication that with oils conforming to the suggested specifications, this concentration is unnecessarily high.

The *petroleum oils* included in Table XV were, with the exception of samples 10 and 11, supplied to specification by various oil refiners. Samples 10 and 11 were used in field trials to determine whether petroleum oils containing aromatic hydrocarbons could be used to replace tar oils for the control of *Aphis*. Both failed for this purpose, a failure associated with the low percentage soluble in dimethyl sulphate. Sample 10 proved inferior to 1-9 for the control of common green capsid, an inferiority associated with its low percentage unsulphonated residue (Austin, Jary and Martin (3)). Sample 11 also failed as a general ovicide in field trials, as would be expected from its low-boiling range and viscosity. Both samples 10 and 11 are excluded from the specified grades; samples 1-9 satisfy the requirements of Grade E oils, samples 1, 3, 4, 6, 7, 8 and 9 fulfil Grade F requirements, whilst samples 3, 4, 6 and 7 conform to the Grade G specification.

It is difficult to quote examples of *tar oil preparations* (Table XVI) conforming to the suggested specifications, for it is apparent from the analyses of typical so-called "standard" tar distillate washes that these products are undergoing a transition from the early type of tar oil "carbolineum" wash, in which the neutral oils were entirely of tar origin, to tar-petroleum preparations containing in some cases up to 40 per cent. of petroleum oils. The majority of the preparations of the

Table XIV.

Tar oil analyses.

Sample No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Neutral oils, % by weight	88.6	89.4	90.6	89.3	82.8	89.6	83.0	89.9	92.3	58.2	91.7	89.5	88.1	92.6	89.6
	88.5	87.9	90.0	—	84.8	—	84.8	88.4	91.4	56.8	—	88.2	88.9	91.2	90.0
Tar acids, % by weight	5.2	5.1	4.0	4.6	4.6	2.3	1.4	5.2	2.7	4.2	2.1	5.0	3.7	0.7	4.4
	5.2	4.5	4.4	—	4.5	—	1.5	4.7	3.1	5.0	—	4.8	3.9	0.9	5.1
Tar bases, % by weight	5.6	—	—	4.9	6.8	—	6.8	6.3	4.2	4.0	6.3	5.7	7.0	7.0	6.0
	5.9	—	—	—	7.0	—	6.0	6.3	4.2	4.3	—	5.7	7.2	7.5	6.0
Neutral oils:															
Sp. gr. (60° F.)	1.089	1.106	1.103	1.086	1.105	1.100	—	1.107	1.097	1.093	1.106	1.112	1.112	1.105	—
Boiling range:															
90 % by volume above (° C.)	248	269	268	248	275	255	—	261	254	273	284	267	291	283	278
50 % by volume above (° C.)	320	335	327	325	333	325	335	328	334	336	345	338	339	340	332
20 % by volume above (° C.)	370	375	366	377	365	359	—	370	373	371	—	379	370	370	373

Table XV.

Petroleum oil analyses.

Sample No.	1	2	3	4	5	6	7	8	9	10	11
Neutral oils, % by weight	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	97.6	100.0
										97.5	
Sp. gr. (60° F.)	0.885	0.903	0.860	0.868	0.900	0.864	0.864	0.885	0.875	1.003	0.864
Boiling range:											
90 % by volume above (° C.)	328	348	312	320	345	325	321	355	326	320	238
50 % by volume above (° C.)	362	378	347	354	366	361	359	390	362	359	291
20 % by volume above (° C.)	392	(394)*	369	390	385	391	386	—	398	394	346
Viscosity (sec.), Redwood I at 70° F.	—	—	125	137	200	136	126	357	157	355	45
Unsulphonated residue, % by volume:											
Method (a)	89.6	—	98.0	97.9	—	97.0	97.0	97.3	—	—	—
	89.7	—	—	—	—	95.2	95.8	96.1	—	—	—
Method (b)	89.4	73.5	—	—	62.6	—	—	—	82.4	14.9	67.1
	90.0	73.7	—	—	63.3	—	—	—	81.3	13.9	65.3
Dimethyl sulphate, % insoluble in	—	—	—	—	—	—	—	—	—	77.0	87.0
										77.0	

* Temperatures placed in brackets are those at which 70 per cent. or less have distilled.

Table XVI.
Tar oil preparation analyses.

Sample No.		1	2	3	4	5	6	7	8	9	10
Type of emulsification		M.O.	M.O.	2-sol.	M.O.	S.E.	M.O.	S.E.	M.O.	—	—
Neutral oils, % by weight		53.5	57.4	81.3	67.5	45.8	70.6	66.0	64.5	67.0	80.0
Tar acids, % by weight		18.9	12.8	1.8	8.4	4.0	5.0	0.7	4.3	12.8	4.5
Tar bases, % by weight		3.1	3.4	6.0	3.7	3.1	1.5	4.5	—	4.0	3.8
Neutral oils:		3.0	—	6.1	4.7	2.8	1.6	4.9	—	—	—
Sp. gr. (60° F.)		0.993	0.981	1.085	1.059	1.081	0.988	1.089	1.034	—	—
Boiling range:		—	200	276	238	277	210	284	249	—	—
90 % by volume above (° C.)		—	237	310	304	316	253	325	325	—	72.7 %
50 % by volume above (° C.)		263	290	340	355	349	315	365	325	—	above
20 % by volume above (° C.)		0.0	—	0.0	7.5	—	7.6	0.0	—	—	270° C.
Dimethyl sulphate, % by volume insoluble in		—	—	—	—	—	—	—	—	—	—
Unsulphonated residue, % by volume		—	0.0	—	7.7	0.0	5.3	—	11.0	—	—
					4.9				12.6		
					5.9				10.6		

Table XVII.
Tar-petroleum oil preparations.

Sample No.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Type of emulsification		M.O.	2-sol.	S.E.	M.O.	M.O.	M.O.	S.E.	S.E.	S.E.	S.E.	2-sol.	S.E.	M.O.	M.O.	M.O.	M.O.
Neutral oils,		62.4	87.2	54.2	71.7	72.0	75.9	64.2	68.8	67.7	71.1	78.8	57.5	62.8	67.6	72.0	70.9
% by weight		62.3	87.6	—	72.5	—	75.5	61.9	67.2	67.6	71.3	78.7	56.9	63.2	66.2	72.6	70.3
Tar acids,		7.9	1.7	3.8	10.3	3.4	6.0	1.8	0.5	1.1	1.6	1.5	1.3	5.6	8.4	6.8	9.1
% by weight		8.3	1.6	—	9.5	—	5.3	1.8	0.6	1.3	1.6	1.7	1.6	5.2	9.0	6.9	9.4
Neutral oils:		1.010	0.980	1.038	0.996	0.985	—	0.958	0.983	0.943	1.005	0.980	0.971	1.017	1.009	1.010	1.003
Sp. gr. (60° F.)		243	300	—	262	234	236	286	294	288	296	279	288	234	248	262	271
Boiling range:		320	370	321	336	293	315	337	333	340	355	334	346	303	304	335	351
90 % by volume above (° C.)		376	—	372	386	371	—	(359)	(353)	—	(385)	(358)	386	366	356	385	388
50 % by volume above (° C.)		33.0	61.1	17.8	41.2	26.4	22.6	61.1	55.0	70.0	50.8	54.0	60.9	18.1	34.8	34.4	42.2
20 % by volume above (° C.)		33.3	64.4	18.4	40.8	25.2	21.2	62.8	54.0	70.0	49.8	54.0	59.7	18.5	34.7	35.5	41.8
Dimethyl sulphate, % by volume insoluble in		—	—	11.2	29.1	14.2	15.9	54.2	46.1	60.6	29.5	43.3	47.7	16.3	26.4	23.5	29.3
Unsulphonated residue, % by volume		—	—	11.4	29.9	15.0	17.4	52.4	44.7	60.0	32.1	43.6	47.7	14.7	28.6	27.5	31.8

Table XVIII.
Petroleum oil preparations.

Sample No.	1	2	3	4	5	6	7	8	9	10
Type of emulsification	...	S.E.	M.O.	S.E.	S.E.	S.E.	S.E.	S.E.	S.E.	S.E.
Neutral oils, % by weight	82.1 81.3	79.7 80.8	85.8 86.8	66.0 65.1	66.3 66.0	66.8 68.6	79.6 80.3	70.8 70.9	56.8 56.6	69.0 70.4
Neutral oils:										
Sp. gr. (60° F.)	0.921	0.887	0.903	0.891	0.919	0.881	0.908	0.895	0.901	0.886
Boiling range:										
90 % by volume above (° C.)	336	369	345	311	324	324	314	360	329	325
50 % by volume above (° C.)	369	399	375	370	358	362	350	—	377	360
20 % by volume above (° C.)	(389)	—	(391)	397	387	(378)	—	—	(398)	389
Viscosity (sec.), Redwood 1 at 70° F.	—	—	—	118	—	—	—	—	—	—
Dimethyl sulphate, % by volume insoluble	—	—	100.0	—	88.6	84.0	—	—	—	100.0
Unsulphonated residue, % by volume	67.1 67.4	78.0 78.9	66.8 68.4	68.7 69.4	88.0 62.2	86.5 63.1	71.4 —	70.8 70.9	76.0 75.4	86.3 86.8
Sample No.	11	12	13	14	15	16	17	18	19	
Type of emulsification	...	S.E.	S.E.	S.E.	S.E.	S.E.	S.E.	M.O.	M.O.	
Neutral oils, % by weight	70.8 70.2	68.3 69.2	69.6 69.5	79.9 80.3	70.9 70.0	63.8 65.2	67.6 66.3	81.4 80.0	84.8 84.0	
Neutral oils:										
Sp. gr. (60° F.)	0.879	0.883	0.866	0.865	—	0.830	0.995	0.921	0.894	
Boiling range:										
90 % by volume above (° C.)	331	325	314	316	316	221	323	343	250	
50 % by volume above (° C.)	365	362	354	351	356	246	360	382	325	
20 % by volume above (° C.)	399	392	—	—	—	268	395	(391)	367	
Viscosity (sec.), Redwood 1 at 70° F.	140	—	—	—	—	35	—	—	—	
Dimethyl sulphate, % by volume insoluble	—	—	—	—	—	—	71.7	97.0	—	
Unsulphonated residue, % by volume	88.2 87.4	91.2 91.3	94.4 96.0	96.3 96.5	94.2 93.2	78.1 80.8	73.5 22.3	—	—	

Long Ashton type would conform to the Grade A specification, e.g. sample 7 of Table XVI, which was placed on the market as a modified Long Ashton wash, though sample 3, which was a commercial brand of two-solution Long Ashton wash contains neutral oil fulfilling the requirements of a Grade B oil. Sample 4, taken from a consignment supplied to the grower as a special wash, conforms to the Grade B type M.O. specification. Samples 1, 2, 5, 6 and 8 are all from preparations placed on the market and fail to come within any of the suggested specifications; the following comments may explain their exclusion. Samples 1 and 2 were submitted by growers for examination and it is not known whether they were recommended by the makers as winter washes. In both samples the presence of relatively large amounts of tar acids reduces the neutral oil content below requirements, and the tar oil present is of too low a boiling range. Sample 6 is of interest in that it proved, in field trials (Austin, Jary and Martin⁽¹⁾), almost useless for the control of the capsid *Plesiocoris rugicollis*, as would be expected, because of the low boiling range of the neutral oils present. Sample 8, which is a well-known and successful winter wash, contains too much petroleum oil to permit of its classification as a tar oil preparation. It is suggested that an improvement in its general utility would be shown if the preparation were modified to conform to the Grade C specification.

Preparations 9 and 10 are quoted from Beran and Watzl⁽⁴⁾; they were included as general tests, carried out at the Bundesanstalt für Pflanzenschutz, and showed that 4–5 per cent. of neodendrin (sample 10) was as effective or only slightly less effective than dendrin (sample 9), a result in agreement with the conclusion that the efficiency of hydrocarbon oil preparations is governed by their content of high-boiling neutral oils. Sample 10 also serves to illustrate the need for greater knowledge by applied biologists of the composition of proprietary washes used for experimental work, since Beran and Watzl appear to accept the preparation as a tar-distillate wash, yet Colizza (abst. in *Rev. appl. Entom.* 1933, **xxi**, 441) stated that neodendrin consisted of 85 per cent. paraffin (*i.e.* petroleum) hydrocarbons and 15 per cent. of solvents.

Of the *tar-petroleum oil preparations* (Table XVII) only samples 2 and 7–12 were stated by the manufacturers to contain petroleum oils. Of these samples 9 and 10, which were marketed especially for the control of capsid, conform to the Grade D, type S.E. specification, except that sample 10 contains insufficient saturated hydrocarbons and consequently fails on the score of low unsulphonated residue. Sample 2 was prepared by an insecticide manufacturer to specification and was successfully used

by several growers for the control of common green capsid on currants. The neutral oil present conforms to the Grade D specification. Sample 7, a successful proprietary wash, and sample 8 conform approximately to the Grade D, type S.E. specification. Sample 11, a commercial two-solution preparation, contains oil satisfying Grade D requirements, except that it is of slightly lower boiling range, whilst sample 12 is unsatisfactory on account of its low oil content.

Of the remaining samples of Table XVII, namely Nos. 1, 3-6, 13-16, all are typical so-called "standard" tar oil washes with the exception of No. 3 which was introduced for use with hard or saline water, but which is of too low an oil content to satisfy requirements. These preparations all contain petroleum oil and yield neutral oils of properties intermediate between those of Grade B and C oils, yet conforming to neither. As all have proved more or less successful in practice as general winter washes, it is important that adequate reasons be given for the failure of these products to come within the specified grades. One reason, common to samples Nos. 1, 4-6, 13-16, is an insufficient content of neutral oils, an excessive amount of tar acids and a low initial boiling range. It would appear that the tar oil present in these samples is of a boiling range which critical experiment has shown less effective as a general ovicide than the type of oil envisaged in the derivation of the Grade C specification. The lower boiling tar oils, *e.g.* Grade B oils, contain less neutral oils and more tar acids than the higher boiling oils, *e.g.* Grade A oils. The scientific evidence available consistently and invariably indicates that the general ovicidal properties of these preparations would be improved if the manufacturers could, without encountering difficulties in the preparation of suitable miscible oils, substitute Grade A tar oil for the lower boiling tar oils at present used. With many of the samples this modification would automatically increase neutral oil content and boiling range and decrease tar acid content to an extent sufficient to bring the preparations within Grade C specification in so far as these items were concerned.

A second reason, illustrated particularly by samples Nos. 5, 6, 13-15, is that the proportion of petroleum oils present is too small to ensure the full utilisation of the advantages gained by the use of petroleum oil in a general wash. There is abundant evidence that, for general ovicidal purposes other than the control of aphid and psyllid eggs, the high-boiling petroleum oils are superior to tar oils. The concentration generally recommended for application with the preparations under discussion is of the order of 7.5 per cent. and, at this concentration, the percentage

SCHEDULE OF PROPOSED SPECIFICATIONS.

(i) Oils.

	Tar oils			Petroleum oils		
	Grade A	Grade B		Grade E	Grade F	Grade G
Neutral oil, % by weight, not less than	88	75		100	100	100
Solid matter, % by weight, not greater than	5	5		—	—	—
Alkali	Nil	Nil		Nil	Nil	Nil
Characteristics of neutral oil:						
Sp. gr. (60° F.)	1.09-1.11	1.05-1.11		0.86-0.92	0.86-0.92	0.86-0.92
Boiling range:						
< 90 % by vol. to distil above (° C.)	270	230		315	310	300: < 10 % by vol. to distil below 330° C.
< 50 % by vol. to distil above (° C.)	325	290		350	345	340: < 50 % by vol. to distil below 365° C.
< 20 % by vol. to distil above (° C.)	365	335		380	375	370: < 80 % by vol. to distil below 390° C.
Viscosity (sec.), Redwood 1 at 70° F.:						
Not less than	—	—		125	100	
Not more than	—	—		500	400	
Unsulphonated residue, % by vol., not less than	—	—		60	80	
Dimethyl sulphate, solubility in	Completely soluble	Completely soluble		—	—	

of neutral oil soluble in dimethyl sulphate need not exceed 47 per cent. to give full control of aphid and psyllid eggs. This conclusion is probably the scientific justification of the gradual and apparently empirical changes of composition which the standard tar oil washes have undergone during the past few years. It is therefore reasonable to suppose that the preparations under discussion represent transitional stages in the evolution of washes which would ultimately conform to the Grade C specification in respect of percentage unsulphonated residue and dimethyl sulphate solubility.

This gradual modification of the "standard" tar oil washes has been accompanied by no indication, on the part of the manufacturer, of any change in composition. Although it is possible that the manufacturer is reluctant to modify the name of his product through fear of loss of goodwill, it is obvious that, from the advisory biologist's point of view, it is most unsatisfactory that such changes in composition should be made without notice or indication. To the grower, the change certainly gives a wash better suited for general ovicidal purposes, but, in many cases, he requires a wash for use solely for the control of aphid and psyllid eggs. In such circumstances a relatively high concentration of the present-day "standard" tar oil washes is required to give a sufficient content of neutral oils soluble in dimethyl sulphate, and the petroleum oil present becomes an expensive waste.

It is for these reasons that the failure of the samples of the "standard" tar oil washes given in Table XVI to conform to a suggested specification need not cause apprehension. On the other hand, it is hoped that the suggestion of separate Grade A, B and C specifications will result in the introduction of manufactured products more suited to the varied practical needs than the present "standard" tar oil washes.

To simplify a discussion of the *petroleum oil preparations* quoted in Table XVIII, the examples have been grouped according to the grade of neutral oil present. Samples Nos. 1-15, of which Nos. 2, 3, 5-10, 14 and 15 have been placed on the market, are of the Grade E type, and all, with the exception of Nos. 4 and 9, satisfy the requirements of this specification, the two exceptions failing on the score of low oil content. The examples of preparations containing the intermediate Grade (F) of oil, samples 10 and 11, both conform to the specification suggested for such preparations. Similarly the examples of preparations (samples 12-15) containing the summer grades of petroleum oil satisfy the requirements suggested for Grade G preparations. Of the remaining materials quoted in Table XVIII, sample 16 was from an experimental

wash intended for early spring application; sample 17, and also samples 5 and 6, represent attempts to prepare general winter washes from petroleum oils only. Samples 18 and 19 are proprietary products of American origin, the former being sold on the basis of a content of 96 per cent. active ingredients. Analysis shows this product to be a typical miscible oil preparation containing beta-petroleum sulphonic acids as emulsifier and approximately 80 per cent. of neutral oils which, strictly speaking, constitute the active ingredients. Sample 19 is sold as a nicotine "activator" and the low boiling range of the neutral oils present indicates that it is of doubtful value as an active insecticide.

Of the preparations described in Table XVIII which have been used in field trials, samples 4, 11 and 16 were applied for the control of *Plesiocoris rugicollis* on apples at the period between dormancy and blossoming. Shewell-Cooper (*in litt.* 17. v. 33) found sample 16 useless, as would be expected from the low boiling range of the neutral oils present, and samples 4 and 11 effective, sample 4 causing injury to blossom and leaves, a phytocidal activity to be associated with the low percentage unsulphonated residue. Washes Nos. 6 and 17 failed as general winter washes (Austin, Jary and Martin⁽³⁾) because they did not adequately control aphids or psylla, a result attributable to the low content of neutral oil soluble in dimethyl sulphate. Sample 15 is of interest, as this material has been found successful for the control of red spider on oil-resistant glasshouse plants.

Finally, the samples of the stock emulsion type of preparation illustrate the need for two grades according to oil content, samples 2, 7 and 14 containing approximately 80 per cent. of neutral oil, whereas samples 4, 6, 8, 10, 11, 12, 13, 15 and 17 fall into the S.E. II group with a permissible minimum of 66.7 per cent. neutral oils.

ANALYTICAL METHODS.

Sampling.

Agitate the contents of the drum by vigorous rolling and, if possible, by stirring until thoroughly mixed. Withdraw samples by inserting slowly a wide glass or metal tube to the bottom of the drum. Close the upper end of the tube and withdraw from the drum, transferring the contents to a convenient bottle or container. Sample at least one drum per four of the consignment, taking at least 1 quart per 40-gallon drum.

Content of solid matter.

With tar oils or tar oil preparations of the miscible oil type, the content of adventitious solids and solid anthracenoid hydrocarbons is determined by filtering a known weight of about 100 gm. of the sample through a weighed filter funnel in the stem of which a small plug of glass wool has been placed. After draining overnight the funnel is reweighed and the weight of solid matter determined.

In some cases the anthracenoid hydrocarbons separating from tar oil preparations on storage may cake to a firm deposit, of which a uniform dispersion cannot be obtained by stirring. If sufficient in amount to warrant examination the drum should be drained through a suitable strainer and the volume of liquid determined. If less than 95 per cent. of the reputed volume it shall be assumed that the tar oil or preparation contains more than 5 per cent. solid matter.

The filtered oil or preparation is used for the subsequent examination of neutral oil content, etc.

Neutral oil content.

Transfer weighed aliquots (75–100 gm.) of the sample to large (1500–2000 ml.) separating funnels with about 500 ml. water. Add 100 ml. of 10 per cent. sodium hydroxide solution and shake with about 500 ml. ether (*d.* 0.730). Stand until a clear ether layer has separated (if the emulsion fails to break sufficiently after standing overnight, add a little (25–50 ml.) saturated sodium chloride solution and rotate gently) and withdraw the lower dilute emulsion or aqueous layer. Re-extract this with successive lots (200 ml. each) of ether until no further oil is extracted. Unite the ether layers, concentrate to about 500 ml. on a water bath, and wash with 1 per cent. sodium hydroxide until the ready separation of a colourless aqueous layer occurs. Unite and reserve the aqueous layer and washings (*a*).

Extract the combined ether solutions with successive amounts of 4 per cent. hydrochloric acid until the acid layer is no longer strongly coloured. Wash the combined acid extracts with successive amounts of ether until the ether layer is colourless. Reserve the combined acid layers (*b*).

Unite the ether washings with the main ether extract and concentrate to 250–300 ml. on a water bath. Add about 20 gm. anhydrous sodium sulphate. After 24 hours, filter into a weighed 250 ml. extraction flask and wash the sodium sulphate and filter with ether (dried over anhydrous sodium sulphate), collecting the excess of filtrate in a suitable

flask. Distil off the ether on a water bath, adding the excess of filtrate during the process, and remove the last traces of ether by placing the flask in a steam oven and drawing a gentle stream of air over the surface of the oil until the smell of ether is no longer apparent. When cool, weigh flask and oil and calculate the percentage by weight of neutral oil.

Note. Certain emulsifiers, *e.g.* beta-petroleum sulphonic acids, are relatively insoluble in 2 per cent. sodium hydroxide, and are not removed by the above procedure. Such emulsifiers are detected by the formation of a definite intermediate layer during ether extraction or by the formation of an emulsion when one or two drops of the extracted oil are shaken with 5 ml. water. The extraction must then be repeated, avoiding the addition of sodium hydroxide in amounts sufficient to give more than a 0.1 per cent. solution. The combined ether extracts are finally extracted with 1 per cent. sodium hydroxide and the analysis continued as above.

Tar bases content.

Add excess of concentrated sodium hydroxide solution to the combined acid washings (*b*), and, when cold, extract with successive amounts of ether until the final ether extract is colourless. Dry the combined ether extracts and determine, as above, the percentage by weight of tar bases.

Tar acid content.

Remove, by distillation, the ether dissolved in the combined sodium hydroxide washings (*a*), and transfer the washings to a beaker. Add solid barium hydroxide and place on a boiling water bath until the precipitate (*c*) has coagulated. Add a few drops of barium chloride solution when the non-formation of a further precipitate will indicate a complete removal of fatty acid, resin or sulphonic acid derivatives present as emulsifier. Filter hot through a Buchner funnel with suction. Acidify the filtrate with concentrated hydrochloric acid and, when cold, extract with successive amounts of ether. Dry the combined ether washings with anhydrous sodium sulphate. After 24 hours, filter into a weighed extraction flask and, after removal of the ether, reweigh and calculate the percentage by weight of tar acids.

Note. Treatment with barium hydroxide may be omitted if no emulsifier is present, *e.g.* with straight tar oils, when the tar acids may be extracted direct from the sodium hydroxide washings after acidification.

Examination of emulsifier.

A heavy barium precipitate (c) is indicative of soap, resin or sulphonic acid emulsification, and confirmation may be obtained by warming the precipitate with dilute hydrochloric acid and extraction, when cold, with ether. The combined ether extracts are washed with water until the aqueous layer no longer has an acid reaction. Dry the ether extract with anhydrous sodium sulphate, filter and evaporate off the ether. The nature of the emulsifier (fatty acid, sulphonated fatty acid, resin or sulphonic acid) may be determined by the usual methods.

The suitability of preparations of the stock emulsion type for use in combination washes (Type S.E. IIb) is determined by the absence of alkali or ammonium salts capable of yielding insoluble calcium or lead salts when mixed with lime sulphur or lead arsenate. Such ammonium salts are detected by the evolution of ammonia on agitation, whilst the alkali salts are detected by ashing a small sample of about 10 gm. in an evaporating basin after expelling water on a water bath. An ash which dissolves in water to give a solution which shows persistent alkalinity to phenolphthalein after the removal of any lime present by passing carbon dioxide through the solution, indicates the presence of an emulsifier unsuitable for the S.E. IIb type of preparation.

Examination of neutral oils.

A sufficient amount of neutral oils is obtained by combining the residues from duplicate determinations of neutral oil content.

Specific gravity. Determine by means of 25 ml. specific gravity bottle, correcting to 60° F. For details see *Standard Methods of Testing Petroleum and its Products*, Institution of Petroleum Technologists, 2nd ed. 1929, p. 1.

Viscosity. Determine with the Redwood No. 1 viscometer at 70° F. in accordance with the standard method (I.P.T. L.O. 8).

Boiling range. Transfer 100 ml. neutral oil to standard distillation flask and distil according to procedure given in *Standard Methods for Testing Tar and its Products*, Standardisation of Tar Products Test Committee, 1929, p. 168. To check the temperatures at which 10, 50 and 80 per cent. by volume have distilled over, it is sufficient to collect the distillate in a 100 ml. measuring cylinder, recording the temperature of the thermometer when 10, 50 and 80 ml. have collected. The temperature of distillation must be so adjusted that the distillate collects at the uniform rate of two drops per second.

Typical figures for distillation range determined by this method, when transferred to squared paper, give smooth S-shaped curves (see *e.g.* Fig. 1, p. 347), and, in general, at least three points separated as widely as possible are sufficient to define the curve. One of these points is obviously at 50 per cent., whilst the other two should be as far apart as possible but not on those parts of the curve which approach asymptotically the 0 and 100 per cent. distillation ordinates. Further, in the case of the higher temperature, it is better that this should not exceed that (approximately 370° C.) above which pyrolysis becomes important. It is therefore suggested that the temperatures at which 10, 50 and 80 per cent. by volume distil be taken to define the boiling range of an oil, but that, as the content of high-boiling fractions is required, these temperatures be expressed as those above which 90, 50 and 20 per cent. of the oil distils.

Percentage unsulphonated residue.

(a) With oils of 60 or more per cent. by volume unsulphonated residue. Transfer to a Chancel's sulphurimeter a volume of the neutral oil sufficient to reach approximately to the 30 per cent. mark, reading the volume at the upper meniscus. Add twice this volume of concentrated sulphuric acid (*d.* 1.84) and shake vigorously for 2 min., loosening the stopper from time to time to permit the escape of sulphur dioxide. Place the sulphurimeter in a boiling water bath so that the level of the water is above that of the oil-acid mixture in the tube. After about 5 min. remove and invert the tube at least twice. Release stopper and replace in the water bath. Repeat this process at least twenty times and finally remove the tube, placing it in an upright position. When cold, read off the volume of the supernatant unsulphonated residue and calculate to percentage of initial volume of oil taken.

(b) If tar oils or oil of unsulphonated residue less than 50 per cent. are present, solid sulphonation products interfere with the method (a). With such oils, dilute a known volume of oil in a stoppered measuring cylinder with an equal volume of benzene, add two volumes of concentrated sulphuric acid (*d.* 1.84) and shake, cooling under water if the temperature rises above 30° C. Stand overnight and read off the volume of the residual supernatant layer. Withdraw a sufficient amount and transfer to a Chancel's sulphurimeter, proceeding with the sulphonation by the method (a).

Notes. (1) If x =initial volume of oil taken and y =volume of oil-benzene layer after the preliminary sulphonation; and if a =volume

transferred to Chancel's sulphurimeter (in Chancel percentage figures) and b = residual volume of unsulphonated oil, the percentage by volume unsulphonated residue = $\frac{100by}{ax}$.

(2) If the residual oil layer in method (a) or the oil-benzene layer in method (b) is too dark in colour to allow the easy demarcation of the oil-acid interface, add a small quantity of water gently from a wash bottle so as to form an intermediate layer.

Dimethyl sulphate, percentage insoluble in.

To one volume of neutral oil in the Chancel's sulphurimeter add two volumes of dimethyl sulphate. Shake vigorously for 1 min. and stand upright overnight. Read off the volume of the supernatant layer and calculate its percentage of the original volume of oil taken. The temperature should not exceed 20° C.

Notes. (1) Because of the dark colour of the oils it is necessary to read volumes at the upper meniscus.

(2) Old samples of dimethyl sulphate may give unreliable results.

Alkali content.

This item, which applies only to oils intended for the home preparation of washes by the two-solution method, is included, as the presence of alkali in the oil may lead to faulty emulsification through interaction with the emulsifier. About 10 ml. of the oil are shaken with an equal volume of water which should show no alkalinity to phenolphthalein after the addition of 1 ml. $N/10$ acid.

EVIDENCE IN SUPPORT OF PROPOSED METHODS OF ANALYSIS.

Oil content.

The proposed method was devised (Martin⁽³⁷⁾) as suitable for the examination of all types of manufactured product coming within the range of the suggested specifications, whether of mixed or separate tar and petroleum oils, or whether of the stock emulsion or miscible oil type. Previous work has dealt with specific types of product, and there is thus little direct evidence upon the suitability of the proposed method to be obtained from the literature. A survey of the methods previously suggested serves, however, to give data upon general or specific points.

The analysis of petroleum oil preparations was the subject of co-operative work by the Association of Official Agricultural Chemists, and reports of this work were published in the *Journal* of that Association

for 1925-7. For the preliminary examination, alternative methods were suggested for the determination of oil content of commercial preparations which were classed, for this purpose, as soap and non-soap emulsions.

With soap emulsions, an indirect method involving the separate determination of water, fatty anhydride and alkali content was found unsatisfactory. The direct method, finally adopted as an official method,¹ consisted in transferring a weighed aliquot to a Babcock cream bottle, breaking the emulsion with hot dilute sulphuric acid and measuring the volume of the oil layer after centrifuging. The necessary correction for phenols and fatty acids, which pass to the oil layer in this method, was determined by their separate estimation by light petroleum extraction of the emulsion broken by the addition of alcohol and sodium hydroxide, the fatty acid content of the extract being calculated from the result of titration with alkali.

The following comments seem pertinent: firstly, that with oils of low unsulphonated residue, the sulphuric acid treatment requires modification; secondly, that errors are introduced if the molecular weights of the acid derivatives of the emulsifier and phenols present differ from that of oleic acid.

No official recommendation followed the co-operative work upon methods suitable for non-soap preparations, but the State of California Department of Agriculture (Marshall⁽³⁶⁾) has adopted somewhat similar methods to these examined by the Association of Official Agricultural Chemists for the analysis of such products. In these methods a weighed aliquot of the preparation is transferred to a Babcock cream bottle and the suitable method of breaking the emulsion determined by trial. For gum emulsions concentrated sodium hydroxide, for emulsions of the Pickering type solid sodium carbonate, and for other types barium hydroxide with sodium hydroxide, are employed to break the emulsions, the volume of the resultant oil layer being determined after whirling in a centrifuge.

According to Swingle and Snapp⁽⁶⁷⁾, these methods are not satisfactory for certain emulsifiers, and these workers employed the following procedure for caseinate emulsions. The emulsion was diluted with an equal volume of ether and broken with 20 per cent. sodium hydroxide in a separating funnel. After standing until the ether layer had separated, it was withdrawn, the ether evaporated off and the volume of residual oil measured. They reported that one ether extraction was sufficient for the complete removal of oil.

¹ See *J. Ass. Off. agric. Chem.*, Wash., 1928, **XI**, 64.

The main reason for the adoption of centrifugal methods by the Association of Agricultural Chemists and by the State of California Department of Agriculture to the exclusion of extraction methods appears to be that the accepted method should be applicable to preparations containing kerosene, with which a loss of oil would occur during the removal of the solvent if an extraction method were used. The specifications to which the method proposed here applies are, however, all for high-boiling oils, and this objection to an extraction method does not arise.

Of the possible reagents which could be used to break the emulsion, sodium hydroxide has the advantages of causing a rapid breaking of all preparations of the miscible oil type, though with certain types of stock emulsion prolonged waiting may be necessary. Further, in preparations containing tar oils, it serves to remove tar acids, a necessary preliminary to the extraction of tar bases. No case has yet been encountered of the failure of the sodium hydroxide method except with miscible oil preparations containing beta-petroleum sulphonic acids as emulsifiers. In such cases the breakdown of the method is quickly apparent, and the only modification required is in the amount of sodium hydroxide solution used.

Previous work on the analysis of tar oil preparations has been concerned mainly with the tar oil disinfectants and the proposed method is, in principle, similar to the methods adopted for this purpose and the modification suggested by Houben⁽²⁷⁾ for the analysis of spray carbolineum preparations. Profft⁽⁵²⁾ employed a distillation method, but this method requires modification to be suitable for preparations of the stock emulsion type. Beran⁽⁴⁾ found the distillation method failed even for carbolineum preparations, as water and emulsifier interfere.

Tar bases content.

The proposed method is similar to that employed by Houben⁽²⁷⁾ and by Profft⁽⁵²⁾.

Tar acid content.

For creosote oils, the Tar Products Tests Committee have standardised a method for the estimation of tar acid content of creosote oils. The oil distilling below 315° C. is treated with strong sodium hydroxide solution, and the volume of tar acids released by the acidification of the alkali solution determined. This procedure could be applied, if required, to the examination of the high-boiling tar oils isolated by the proposed method. As, however, the tar acid content of tar oils decreases as the

boiling range of the oil increases, there would appear to be no reason to require the additional treatment of the extracted neutral oil.

The method by the *Industrieverbände für Pflanzenschutz* (1928) of estimating phenol content by the increase in volume of a caustic soda solution after shaking with the tar oil was found by Profft to be inaccurate and he abandoned it in favour of the proposed method.

Boiling range.

In the absence of a standard distillation method for mineral lubricating oils approved by the Institution of Petroleum Technologists, it is suggested that requirements will be adequately met by the adoption of the apparatus and distillation rate specified by the Standardisation of Tar Products Test Committee.

Unsulphonated residue.

A co-operative examination of a number of suggested methods was carried out by the Association of Official Agricultural Chemists which finally adopted that proposed by Graham⁽¹⁸⁾ as an official method applicable to mineral oils and the oil recovered in the analysis of mineral oil-soap preparations. In this method the oil is shaken under specified conditions with 38*N* sulphuric acid at 60–65° C.

The other methods examined co-operatively differed but little from this method, either 37*N* or 38*N* was used at sulphonation temperatures of either 60–65 or 100° C. From the comments and results of the contributing analysts there would appear to be little difference in the relative merits of the methods, and it may be pointed out that the use of 37*N* acid at 100° C. has been adopted by the State of California Department of Agriculture (Marshall⁽³⁶⁾). Carbonisation of the oil was more pronounced when sulphonated at 100° C., at which temperature slightly higher results were obtained, presumably through a solution of sulphonation products in the oil. The proposed method (*a*) (see p. 401) would appear to evade these disadvantages of the higher sulphonation temperature, and to possess the advantage that the cumbersome preparation of fuming 38*N* sulphuric acid is avoided. Further, since no method known gives an absolute figure for the content of saturated hydrocarbons, an elaborate method is unnecessary provided concordant results are given by a simple method.

For the examination of oils which blackened on sulphonation so that the determination of residual oils was impossible, Graham⁽¹⁸⁾ recommended dilution with an oil of known sulphonation value. Dilution with

benzene of 0 per cent. unsulphonated residue seems an obvious alternative and its use, in method (b), may be justified on the grounds that the results obtained are similar to those given by method (a) with oils for which the latter method is suitable (see *e.g.* Table XV). Further the results obtained by the use of method (b) with tar-petroleum oil mixtures agree with sufficient accuracy with those calculated from the percentage unsulphonated residue of the petroleum oil used. Thus the first four mixtures of strained anthracene oil or high-boiling neutral tar oils (4 parts by volume) and various petroleum oils (6 parts by volume) examined gave the following percentages by volume unsulphonated residue, the theoretical value ($=0.6 \times$ percentage unsulphonated residue of the petroleum oil present) being given in brackets:

- | | |
|-------------------------|-------------------------|
| (1) 56.5 : 55.3 (58.0). | (3) 37.2 (38.8). |
| (2) 42.3 : 43.0 (44.2). | (4) 38.0 : 38.2 (38.8). |

Dimethyl sulphate, percentage insoluble in.

The use of dimethyl sulphate for the quantitative estimation of tar oils in mixtures of tar and petroleum oils has been adversely criticised by Harrison and Perkin^(24a). Their objections appear to be due to the fact that certain petroleum oils show partial solubility in dimethyl sulphate, a fact which, in the case of high-boiling petroleum oils, may be due to the presence of aromatic hydrocarbons. That low-boiling petroleum oils free from aromatics may be slightly soluble in dimethyl sulphate does not affect the case of the high-boiling oils under consideration. The seriousness of Harrison and Perkin's objections is therefore decreased if the purpose of the dimethyl sulphate solubility test be accepted as an approximate quantitative measure of the content of aromatic derivatives in mixtures of high-boiling neutral oils from tar and petroleum sources. The degree of accuracy of the test may be illustrated by the results obtained with the four tar-petroleum oil mixtures referred to above. As the petroleum oils used were of negligible solubility in dimethyl sulphate, and the tar oils were completely soluble, the theoretical percentage insoluble in dimethyl sulphate was 60.0 per cent. The results obtained were:

- | | |
|------------------|------------------|
| (1) 60.7. | (3) 60.7 : 60.1. |
| (2) 58.2 : 63.6. | (4) 61.9 : 61.1. |

SUMMARY.

1. Preparations of certain hydrocarbon oils of either tar or petroleum origin have found wide use in horticulture as insecticides, the oil functioning as an ovicide, an acaricide or a scalecide. Critical experiment supplemented by general experience has shown that the suitability of the oils for these purposes is determined by certain physical and chemical criteria which, collectively, constitute a specification. Previous work on the subject is reviewed and a series of specifications is suggested.

2. Because of the several purposes for which tar and petroleum oils are used, a minimum of five grades of the oils themselves, two of tar and three of petroleum oils, is necessary to cover all requirements.

3. *Tar oils* suitable for the preparation of spray fluids are covered by Grades A and B, which are defined as follows:

Grade A. The oil should contain not less than 88 per cent. by weight of neutral oils, not more than 5 per cent. by weight of solid matter, and should be free from alkali, as determined by the prescribed methods of analysis. The neutral oil isolated by the prescribed method should have a specific gravity of 1.09–1.11 (60° F.), should be completely soluble in dimethyl sulphate and should be of boiling range such that at least 90 per cent. by volume distils above 270° C., at least 50 per cent. above 325° C. and at least 20 per cent. above 365° C.

An oil fulfilling these requirements will be suitable for the preparation of winter washes intended for general purposes.

Grade B. The oil should contain not less than 75 per cent. by weight of neutral oils, not more than 5 per cent. by weight of solid matter and should be free from alkali. The neutral oils, isolated by the prescribed method, should have a specific gravity of between 1.05 and 1.11 at 60° F., should be completely soluble in dimethyl sulphate, and should be of boiling range such that at least 90 per cent. by volume distils above 230° C., at least 50 per cent. above 290° C. and at least 20 per cent. by volume above 335° C.

An oil satisfying this specification will be suitable for the preparation of washes intended for the control of aphid and psyllid eggs by application during the dormant season.

4. *Petroleum oils*, described as Grades E, F and G, should consist entirely of neutral oils, should be free from alkali, and should have a specific gravity of between 0.86 and 0.92 at 60° F.

Grade E. The neutral oils should have a viscosity of between 125 and 500 sec. Redwood 1 at 70° F., should have a boiling range such that not

less than 90 per cent. by volume distils above 315°C ., at least 50 per cent. above 350°C . and not less than 20 per cent. above 380°C ., and should have an unsulphonated residue of not less than 60 per cent. by volume, all figures being determined by the prescribed methods of analysis.

An oil fulfilling these requirements will be suitable for the preparation of winter washes or of washes intended for application at certain periods intermediate between bud burst and blossoming on varieties of fruit tolerant to petroleum oils less highly refined than Grade F oils.

Grade F. The neutral oils should have a viscosity of between 100 and 400 sec. Redwood 1 at 70°F ., should have a boiling range such that at least 90 per cent. by volume of the oils distils above 310°C ., at least 50 per cent. above 345°C . and at least 20 per cent. above 375°C ., and should have an unsulphonated residue of not less than 80 per cent. by volume when determined by the prescribed methods.

An oil satisfying the above requirements will be suitable for the preparation of washes intended for application at periods intermediate between bud burst and blossoming on varieties of fruit tolerant to petroleum oils less highly refined than Grade G oils.

Grade G. The neutral oils should have a viscosity of between 75 and 150 sec. Redwood 1 at 70°F ., should be of boiling range such that at least 90 per cent. by volume distils above 300°C ., but not less than 10 per cent. by volume should distil below 330°C ., not less than 50 per cent. by volume should distil above 340°C ., and below 365°C ., not less than 20 per cent. by volume should distil below 370°C ., and not less than 80 per cent. by volume should distil below 390°C ., and the unsulphonated residue should not be less than 90 per cent. by volume, all figures being determined by the prescribed methods.

An oil conforming to this specification will be suitable for the preparation of sprays intended for application to foliage of oil-tolerant varieties of plant.

5. The successful use of oils of the above grades is dependent upon proper application in spray form, for which suitable methods of emulsification are necessary. Although methods are available whereby the grower can prepare emulsions of these oils, it is simpler for him to use manufactured oil preparations which merely require mixing with water to give the required spray. Such oil preparations are of two types, miscible oils (designated M.O.) and stock emulsions (S.E.). Miscible oils are clear oily liquids which, on mixture with water, yield emulsions. Stock emulsions are cream-like pastes and, as their name implies, are concentrated emulsions. The amount of neutral oil which can conveniently be incorporated in the preparation is dependent on the type of emulsifier used, and, to

cover all types, four types of preparations are specified. In addition, two further grades of neutral oil, Grades C and D, are necessary to cover combined tar-petroleum oil preparations.

6. The following specifications for tar and petroleum oil preparations cover all present requirements:

Tar oil preparations.

Grade A, Type M.O. The preparation should contain not less than 70 per cent. by weight neutral oils, not more than 4.2 per cent. by weight tar acids and not more than 5 per cent. by weight solid matter, as determined by the prescribed methods.

Grade A, Type S.E. The preparation should contain not less than 60 per cent. by weight neutral oils and not more than 3.6 per cent. by weight tar acids, as determined by the prescribed methods.

The neutral oil, isolated by the prescribed method from both the M.O. and S.E. types, should have a specific gravity of between 1.06 and 1.11 at 60° F., the percentage insoluble in dimethyl sulphate should not exceed 10 per cent. by volume, and the boiling range of the oil should be such that at least 90 per cent. by volume distils above 270° C., at least 50 per cent. by volume above 325° C. and at least 20 per cent. by volume above 365° C.

Preparations conforming to the Grade A, Types M.O. and S.E. specifications will be suitable for application as tar oil winter washes for general purposes.

Grade B, Type M.O. The preparation should contain at least 60 per cent. by weight neutral oils and not more than 9 per cent. by weight tar acids and not more than 5 per cent. by weight solid matter, as determined by the prescribed methods of analysis.

Grade B, Type S.E. The preparation should contain at least 50 per cent. by weight neutral oils, and not more than 7.5 per cent. by weight tar acids, as determined by the prescribed methods.

The neutral oils, isolated by the prescribed method from preparations conforming to the Grade B, Types M.O. and S.E. specifications, should have a specific gravity of between 1.05 and 1.11 at 60° F., the percentage insoluble in dimethyl sulphate should not exceed 10 per cent. by volume and the boiling range should be such that at least 90 per cent. by volume distils above 230° C., at least 50 per cent. by volume above 290° C. and at least 20 per cent. by volume above 335° C.

Preparations conforming to the above Grade B specifications are suitable for application as tar oil winter washes intended for the control of aphids and psyllids.

Tar-petroleum oil preparations.

Grade C, Type M.O. The preparation should contain not less than 74 per cent. by weight neutral oil and not more than 2 per cent. by weight tar acids and not more than 5 per cent. by weight solid matter, as determined by the prescribed methods of analysis.

Grade C, Type S.E. The preparation should contain not less than 62 per cent. by weight neutral oils and not more than 1.5 per cent. by weight tar acids, as determined by the prescribed methods of analysis.

The neutral oil, isolated by the prescribed method from Grade C preparations, should have a specific gravity not less than 0.99 at 60° F., and should have a boiling range such that at least 90 per cent. by volume distils above 280° C., not less than 50 per cent. above 335° C. and not less than 20 per cent. above 375° C. Not more than 53 per cent. by volume of the neutral oil should be insoluble in dimethyl sulphate and the unsulphonated residue should not be less than 32 per cent., all determinations being made by the prescribed methods.

Preparations conforming to the Grade C, Types M.O. and S.E. specifications will be suitable for use as combined tar-petroleum oil winter washes for general application on varieties of plants to which washes of high (e.g. 9 per cent.) oil concentration are injurious.

Grade D, Type M.O. The preparation should yield not less than 75 per cent. by weight neutral oils, not more than 2 per cent. by weight tar acids and not more than 5 per cent. by weight solid matter, when examined by the prescribed methods of analysis.

Grade D, Type S.E. The preparation should contain not less than 63.5 per cent. by weight neutral oils and not more than 2 per cent. by weight tar acids, when determined by the prescribed methods of analysis.

The neutral oil, isolated by the prescribed method from preparations of the Grade D, Types M.O. and S.E. specifications, should be of specific gravity not less than 0.95 at 60° F., should be of boiling range such that not less than 90 per cent. by volume should distil above 290° C., not less than 50 per cent. by volume should distil above 340° C. and not less than 20 per cent. should distil above 375° C., and not more than 70 per cent. by volume of the neutral oil should be insoluble in dimethyl sulphate and the unsulphonated residue should not be less than 42 per cent. by volume, all determinations being made by the prescribed methods.

A preparation conforming to the requirements of the Grade D, Types M.O. and S.E. specifications will yield a combined tar-petroleum oil

winter wash suitable for application for general purposes to varieties of plant tolerant to sprays of high oil content.

Petroleum oil preparations.

Grade E, Types M.O. and S.E. I. The preparations should contain not less than 80 per cent. by weight neutral oil, determined by the prescribed method of analysis.

Grade E, Type S.E. IIa. The preparation should contain not less than 66.7 per cent. by weight neutral oil, determined by the prescribed method of analysis.

Grade E, Type S.E. IIb. The preparation should contain not less than 66.7 per cent. by weight neutral oil and should contain no free ammonia or alkali salts yielding insoluble calcium salts or an ash containing free alkali, when tested by the prescribed methods of analysis.

The neutral oil, isolated from preparations of the Grade E, Types M.O. and S.E. I, IIa and IIb specifications, by the prescribed method, should conform to the Grade E specification of petroleum oils given above (p. 407).

Preparations conforming to the requirements of the Grade E specifications are suitable as petroleum oil sprays for dormant use and for application to foliage of varieties of plants tolerant to sprays containing semi-refined petroleum oils. Preparations of the Grade E, Type S.E. IIb specification are suitable for combination with lead arsenate, lime sulphur or Bordeaux mixture on tolerant varieties of plants.

Grade F, Types M.O. and S.E. I. The preparations should yield not less than 80 per cent. by weight neutral oil, determined by the prescribed method of analysis.

Grade F, Type S.E. IIa. The preparation should contain not less than 66.7 per cent. by weight neutral oil, determined by the prescribed method of analysis.

Grade F, Type S.E. IIb. The preparation should contain not less than 66.7 per cent. by weight neutral oils, should yield an ash containing no free alkali, and should contain no free ammonia, or alkali salts yielding insoluble calcium salts, as determined by the prescribed methods of analysis.

The neutral oils, isolated by the prescribed method from preparations of the Grade F, Types M.O., S.E. I, and S.E. IIa and b, should conform to the specification for Grade F petroleum oils given above (p. 408).

Preparations satisfying the requirements of the Grade F specifications are suitable as petroleum oil washes intended for application to

the foliage of plants tolerant to half-white petroleum oils. Preparations of the Grade F, Type S.E. II*b* specification are suitable for use in combination with lead arsenate, lime sulphur or Bordeaux mixture upon foliage of varieties of plants tolerant to such combined washes.

Grade G, Types M.O. and S.E. I. The preparations should contain not less than 80 per cent. by weight neutral oil, as determined by the prescribed method.

Grade G, Type S.E. IIa. The preparation should contain not less than 66·7 per cent. by weight neutral oil, as determined by the prescribed method.

Grade G, Type S.E. IIb. The preparation should contain not less than 66·7 per cent. by weight neutral oil, and should contain no free ammonia or alkali salts yielding insoluble calcium salts or an ash containing free alkali when examined by the prescribed methods of analysis.

The neutral oils, isolated from preparations of the Grade G specifications, should conform to the specification for Grade G petroleum oil given above (see p. 408).

Preparations satisfying the requirements of the Grade G, Types M.O., S.E. I, II*a* and *b* specifications are suitable as petroleum oil washes for application to the foliage of plants tolerant to petroleum oil sprays. Those of the Grade G, Type II*b* specification are suitable for use in combination with lead arsenate, lime sulphur or Bordeaux mixture for application to the foliage of plants tolerant to such combined washes.

For ease of reference and comparison the various specifications proposed are tabulated in the Schedule given above (see pp. 394–5).

7. Methods of analysis are suggested for the determination of the various criteria required by the specifications and evidence is given of the suitability of the suggested methods.

8. The application of the proposed specifications is illustrated by examples from oils and preparations which have been or are at present used in spray practice.

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PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

ANNUAL GENERAL MEETING of the Association held on Friday, February 15th, 1935, at 2.30 p.m. in the Botanical Lecture Theatre of the Imperial College of Science and Technology, London. The Chair was taken by the President, Dr T. GOODEY.

The following papers were read:

- I. Nitrification by Micro-organisms other than *Nitrosomonas*. By D. WARD CUTLER, M.A. and Miss L. M. CRUMP, M.Sc.
- II. Recent Work on the Biological and Chemical Aspects of Nitrification. By A. S. CORBET, Ph.D.
- III. The Nitrification of Ammonia in the Field and in Laboratory Incubation Experiments. By W. G. E. EGGLETON, B.Sc.

I. NITRIFICATION BY MICRO-ORGANISMS OTHER THAN *NITROSOMONAS*.

By D. WARD CUTLER, M.A. AND MISS L. M. CRUMP, M.Sc.

(*Rothamsted Experimental Station, Harpenden, Herts.*)

THE nitrogen cycle as recorded in text-books is a little misleading, though it is customary to speak of this cycle meaning, thereby, that the nitrogen from organic residues, or that which is fixed from the air, in its turn passes to nitrate through nitrite and so comes back into new plant tissues. Though such a chain of events is true in essentials, it must be remembered that there are many factors which may interfere with the orderliness of the series. For instance, even before the decomposition of organic residues has well begun, they may be consumed by worms and insects and either built up again in their bodies or excreted in the form of simple nitrogenous compounds. The ammonia that is ultimately produced from protein is made by the action of bacteria both on the protein itself and on the simpler nitrogenous compounds produced by its decomposition; but it must be remembered that at every stage only a percentage, though a large one, is free ammonia, the rest of the nitrogen having been turned into bacterial protoplasm.

Similarly, when nitrite has been formed from ammonia it may be used as food for certain bacteria instead of being oxidised to nitrate and, again, the whole amount of nitrate formed will not necessarily go back into plant protoplasm as it may be reduced by bacterial action and return to the cycle at an earlier stage.

Since the first isolation of bacteria from soil it has always been easy to find species which have the power to produce ammonia from nitrogenous organic compounds but,

for many years, no organisms were found which were capable of converting the ammonia into nitrite or nitrate, even though Schloesing and Müntz by their experiments had shown that nitrite was formed by biological agencies and not by purely chemical reaction. A survey of the early literature of nitrite formation yields interesting and frequently contradictory views. Schloesing and Müntz, as a result of their work on the purification of sewage water, stated that since chloroform could stop the whole process of nitrification, not only in sewage practice but also in soil, the process must be due to the activity of organisms which required organic matter in order to form nitrate and, therefore, by implication, also needed it for nitrite production.

As regards nitrite formation from ammonium salts there is grave doubt as to whether this is brought about solely by the action of *Nitrosomonas* and its varieties; in fact, there is abundant evidence in the older literature that this is not the case and, in the past few years, Cutler and Crump at Rothamsted have been able to isolate a large number of species of bacteria which in no way approximate to the characteristics of *Nitrosomonas* and its varieties. It has also been shown that the carbon nitrogen ratio has enormous effect on whether nitrite is built up from ammonium salts or is consumed by different bacterial species.

Natural products, such as urine, give greatly increased nitrite production when compared with organic salts. It can only be regarded as reasonable and according to expectation that, since soil is a universal scavenger, it should have a bacterial population especially suited for dealing with natural products by unspecialised groups of bacteria and not by specialised ones.

II. RECENT WORK ON THE BIOLOGICAL AND CHEMICAL ASPECTS OF NITRIFICATION.

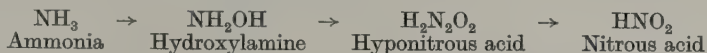
By A. STEVEN CORBET, PH.D.

(*Jealott's Hill Agricultural Research Station, Bracknell, Berks.*)

THE oxidation of ammonia to nitric acid is termed nitrification and Pasteur, in 1862, first suggested that nitrification in soil was a biological process. Proof of this was not forthcoming until fifteen years later, when Schloesing and Müntz in France, and Storer in America, showed that the process was effected by the agency of micro-organisms and could be completely inhibited by heating to 100° C., or by treatment with antiseptics. It was soon realised that nitrification was an oxidation reaction which could proceed only in the presence of adequate supplies of oxygen.

At first all attempts to isolate the organisms responsible for nitrification failed, and it was not until 1890 that Winogradsky, by special culture methods, separated two species of bacteria capable of effecting the oxidation of ammonia to nitrate: *Nitrosomonas* was able to convert ammonium salts to nitrite, while *Nitrobacter* transformed nitrite to nitrate. Both organisms preferred neutral or slightly alkaline media. In spite of the fact that Winogradsky's organisms operated in the absence of organic matter, until quite recently it was believed that the question of soil nitrification was largely, if not entirely, settled. Within the last few years, however, evidence has been adduced showing that purely chemical forces play some part and that, under certain circumstances, it is possible for ammonium salts to be oxidised to nitrate entirely by chemical means.

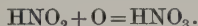
Nitrification by biological agency will be considered first. Some work carried out by the writer (*Biochem. J.* 1934, xxviii, 1575-82) has been directed towards the quantitative determination of the nitrogen compounds present during nitrification by soil micro-organisms, and it has been found that the matter is more involved than was formerly believed. Moreover, under certain conditions, the transformation of ammonia to nitrite takes place in stages, hydroxylamine and hyponitrous acid appearing as intermediate compounds during the reaction:



In the case of the two last-named compounds, intramolecular rearrangement takes place, entailing the elimination of water and production of the compounds as formulated. It has often been suggested that the course of nitrification must be in accordance with the above scheme, for it is the obvious one, but the difficulty has been to detect and isolate the intermediate compounds.

For a full appreciation of the course of nitrification it is necessary to consider the stability of the intermediate compounds. It has been found that hydroxylamine is stable in strongly acid solution, but decomposition sets in when the reaction of the medium is on the alkaline side of 5; it is clear that no accumulation of hydroxylamine can take place under conditions which ordinarily occur in soil or culture media. Moreover, hydroxylamine is unstable in the presence of nitrite; whatever be the pH of the medium, the whole of the hydroxylamine-N and a proportion of the nitrite-N disappear in a remarkably short space of time.

Calcium hyponitrite, in which form hyponitrous acid is most likely to occur in soil and in culture media, is stable in aqueous solution and in the presence of nitrite but, when the medium is warmed, the salt is readily decomposed to give nitrous oxide, nitric oxide and nitrogen. Investigation of the stability of nitrite solutions has shown that oxidation of nitrite to nitrate proceeds by an autoxidation process when the pH of the medium is more acid than 5. It is thus clear that while nitrite accumulation can take place in the usual culture media employed for nitrite-formation studies, conditions are unfavourable in most soils. Nevertheless, nitrite accumulation takes place under acid conditions when the supply of oxygen is inadequate for oxidation to proceed according to the equation:



The biological oxidation of ammonium salts by soil micro-organisms takes place in a variety of ways. The most common case (as judged by soil samples from Jealott's Hill) is that in which the reaction ceases after about 5 per cent. of the ammonia is transformed to nitrite. Subcultures behave in precisely the same manner.

In another type of reaction the ammonium salt is completely oxidised to nitrite, hydroxylamine and hyponitrous acid appearing as ephemeral intermediate compounds during the early stages of nitrification. Again, subcultures behave in exactly the same way, except that repeated subculturing reduces to vanishing point the amounts of hydroxylamine and hyponitrous acid formed. In another type of culture a large proportion of the nitrogen missing during the early stages of nitrification was shown to be present as hyponitrous acid; finally, all the nitrogen appeared as nitrite.

Probably the most interesting biological oxidation is that entailing the oxidation of ammonium sulphate to hyponitrous acid; this latter compound appears to break down, the nitrogen escaping in some form not yet determined. This particular culture,

which gives the same results on repetition, was obtained from a grass plot which had been treated with nitro-chalk some months previously. Hyponitrous acid formation occurred in other cultures of soil micro-organisms from grass plots which had been treated recently with inorganic fertilisers.

Detailed results of these experiments will shortly be published elsewhere.

Recent work suggests that the chemical aspects of nitrification in the soil, hitherto ignored, may be of more than academic interest under certain conditions. It is nearly a quarter of a century ago since Berthelot and Gaudechon first reported nitrite formation from ammonium salts by the action of ultraviolet light, but it is only within the last year or two that it has been seriously suggested that photonitrification may be an important process in soil.

G. Gopala Rao and N. H. Dhar (*Soil Science*, 1931, **xxxI**, 379-84; 1934, **xxxviii**, 143-59) have advanced evidence to show that under certain conditions in tropical soils, nitrification is at least partly due to a photochemical mechanism. They have found that appreciable amounts of nitrite are formed, not only from ammonium salts, but also from certain nitrogenous organic compounds, in the presence of ultraviolet light and a photosensitiser (such as titania). It is pointed out that the nitrifying capacity of soils is at a maximum during the summer and at a minimum in winter, while it is well known that sunlight inhibits the activities of nitrifying bacteria; moreover, the high temperatures prevailing in exposed tropical soils are inimical to bacterial action. The writer reached a similar conclusion, and found that nitrite formation occurs in soils in the absence of added ammonium salts or photosensitisers. In one experiment it was found that after 150 gm. of soil were exposed to the mercury arc for 500 hours, there were present 12 parts per million of nitrite-nitrogen. The soil used (*pH* about 5.8) was one in which no nitrite accumulation normally took place.

A year or two ago G. de Rossi (*Soc. Internaz. Microbiol., Boll. Sez. Ital.* 1933, **v**, 132-6) found that a rapid nitrification occurs in the superficial layers of soil as a result of a physico-chemical process and independent of microbiological activity. The reaction is not due to the action of sunlight, and is intensified at relatively high temperatures. The writer found that alternate wetting and drying out of soil, such as occurs in cleared soils in the equatorial tropics, in the absence of light resulted in accumulation of small amounts of nitrite. After 25 days of such treatment a soil contained 2 parts per million of nitrite-nitrogen.

Reference has been made already to the fact that oxidation of nitrite to nitrate can proceed by a chemical reaction in acid media. This fact accounts for the absence of nitrite from most soils, and it is evident that the complete process of nitrification, entailing the oxidation of ammonium salts to nitrate, can be effected by chemical means.

In the presence of ultraviolet light, nitrates are reduced to nitrite, and the amount of nitrite produced by this process is of the same order as that obtained by the photochemical oxidation of ammonia. It will be clear, however, from previous considerations, that nitrite accumulation can become effective only when the reaction of the medium is above *pH* 5.

It remains to assess the importance of the various nitrification processes described. The fact that nitrite-producing bacteria are almost invariably present in soils in temperate regions, while *Nitrobacter* may often be absent, suggests that although nitrite formation from ammonium salts is usually biological in nature, the further

oxidation to nitrate is effected largely by chemical means. That *Nitrosomonas* occurs in tropical soils is well established, but the evidence presented by the Indian investigators, de Rossi and the writer, indicates that, at least under certain conditions, nitrification in equatorial soils may be attributed to chemical forces. It remains to be shown to what depth photonitrification can occur but, in this connection, it may be mentioned that the depth of the top-soil layer in tropical soils exposed to the sun is considerably less than that found in temperate regions.

Quite recently N. H. Dhar (*Nature*, Lond., 1934, cxxxiv, 572-3) has suggested that denitrification known to take place in soil in the presence of readily oxidisable organic material may be explained as a result of chemical processes. It is supposed that photo-oxidation of ammonium salts to nitrite results in accumulation of ammonium nitrite which, under the action of light and in the presence of a photosensitiser such as titania, decomposes with liberation of gaseous nitrogen. Work by the writer has shown that gas is liberated in small amounts from ammonium nitrite in the presence of freshly ignited titania by the action of sunlight or ultraviolet light, but it seems evident that any appreciable losses of nitrogen from the soil cannot be explained in this way.

III. THE NITRIFICATION OF AMMONIA IN THE FIELD AND IN LABORATORY INCUBATION EXPERIMENTS.

BY W. G. E. EGGLETON, B.Sc.

(*Jealott's Hill Agricultural Research Station, Bracknell, Berks.*)

It has always been a matter of considerable importance to the agriculturist to know exactly what happens to a nitrogenous fertiliser when added to a soil: to be able to follow the various transformations which it undergoes until it finally appears in an agricultural crop. Some of the nitrogen which is added to soil, whether it be added as ammonia or nitrate, is undoubtedly taken up in an unchanged form by the growing plant, but the common experience is that this process is not 100 per cent. efficient. Some of the nitrogen which is applied to the soil does not find its way into the crop for which it was destined—at least during the course of one season. Where then does it go? That is the problem. It is very necessary to know whether that portion is really lost for good and all, or whether it is only temporarily withheld from the crop; it is necessary to know also in what form or forms it exists, whether some or all of this fraction is ever likely to become available to the crop plant, and, if so, whether it is likely to become available suddenly or only gradually. These and a host of other questions still remain to be answered in certain terms.

One thing that must be borne constantly in mind in endeavouring to find a solution to these problems is that the various forms of life, plant and animal, which grow in association in the soil, depend primarily upon soil and climatic conditions, *i.e.* they all depend upon the intensity and incidence of moisture and temperature, upon the abundance of various minerals and so forth, and compete actively one with another for their life requirements. They tend to increase and multiply as the various circumstances permit, or to pass out of existence if conditions are against them. This is, of course, a fundamental ecological fact.

As a necessary consequence of this state of affairs, the soil under natural conditions must be regarded as a system which is at any time not very far from being in a state of equilibrium. It is probably never actually so because the various factors concerned are always changing in some degree, but the point I wish to emphasise is that the tendency must be for those changes (biological and chemical) to take place which make *towards* a state of equilibrium, even though that state may never actually be realised. Thus we know, for example, that the ratio of carbon to nitrogen in soil usually centres ultimately round about 10 or 11, and that if by any means this ratio is widened by the addition of carbonaceous material, nitrate production is suppressed and CO_2 evolution is increased until the *status quo* is reached again, and *vice versa*. We know also that in grassland soil during growing conditions, the amount of nitrate present in the soil is usually very small indeed, small because it is continually being extracted from the soil by the herbage. There is little doubt that nitrate is being actively produced, for in a strictly comparable soil without grass nitrate can be shown to accumulate. Similarly, *the ammonia level is somewhat lower in the presence of grass than in its absence*, at least during the growing season, and again, the number of micro-organisms is greater under grass than in the same soil without grass, and so on.

These are merely a few of the many factors, the resultant of which defines the fertility status of the soil, and I think you will agree that in such a complex system of balanced forces no component, either chemical or biological, however insignificant it may be, can change its value or can enter or leave the system without the effect of such a disturbance being felt in some degree, either directly or indirectly, sooner or later, by all the members of the soil community. Thus, for example, the addition of an amount of soluble nitrogenous salt to the soil system, in raising the N status of the soil, may be expected to bring about a whole series of adjustments, biological as well as chemical. In the course of this process of adjustment to the new N level, those organisms, both plant and animal, which stand in need of nitrogen or are in a position to utilise the nitrogen in the form in which it is applied, immediately compete to the limit of their ability for this sudden abundance of an essential food ingredient. In the struggle, so to speak, the grass gets its share, which may be indeed the lion's share, but that some at least finds its way into the body substance of the competing soil microflora, and thus indirectly into the microfauna, appears equally certain.

I have dwelt upon this aspect of the subject because it is against such a background that I think the few results which I am presenting should be considered. *In soil we have an actively competitive heterogeneous population, which is always pressing hard upon its means of subsistence.*

In Fig. 1 I have plotted data derived from an experiment conducted at Jealott's Hill in 1932. In the top section I have shown the excess numbers of bacteria in the top 4 in. of plots of grassland which had been treated with 2 cwt./acre sulphate of ammonia, over corresponding plots which had received no nitrogen. The numbers refer to plate counts. You will notice that the application of nitrogen in March has resulted in a marked increase in numbers of bacteria on both unirrigated and irrigated soil. The increase on the unirrigated soil corresponds to about 20 millions/gm. dry soil on a mean count of about 35 millions. On the irrigated soil the increase represents about 25 millions on a mean count of about 60 millions. These increases refer, as I have said, to plate counts, but it is interesting to note that from what little is known regarding the ratio of plate counts to absolute numbers, these increases represent a

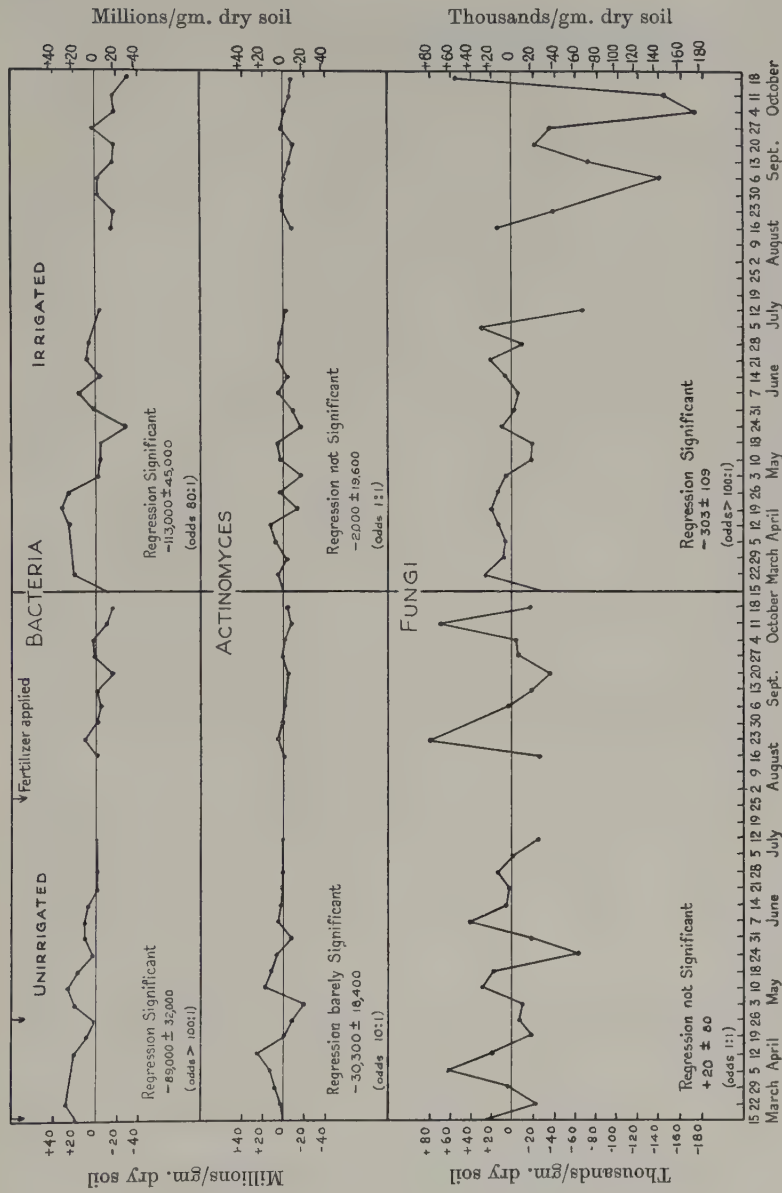


Fig. 1. Excess numbers of bacteria, actinomycetes and fungi on nitrogen-treated plots over the corresponding numbers on the plots receiving no nitrogen. Whiskers Field, 1932.

utilisation by bacteria of something like 5 lb. out of the 46 lb. of nitrogen which were applied, *i.e.* something over 10 per cent. I mention this merely because it gives a very rough idea of the amount of nitrogen involved.

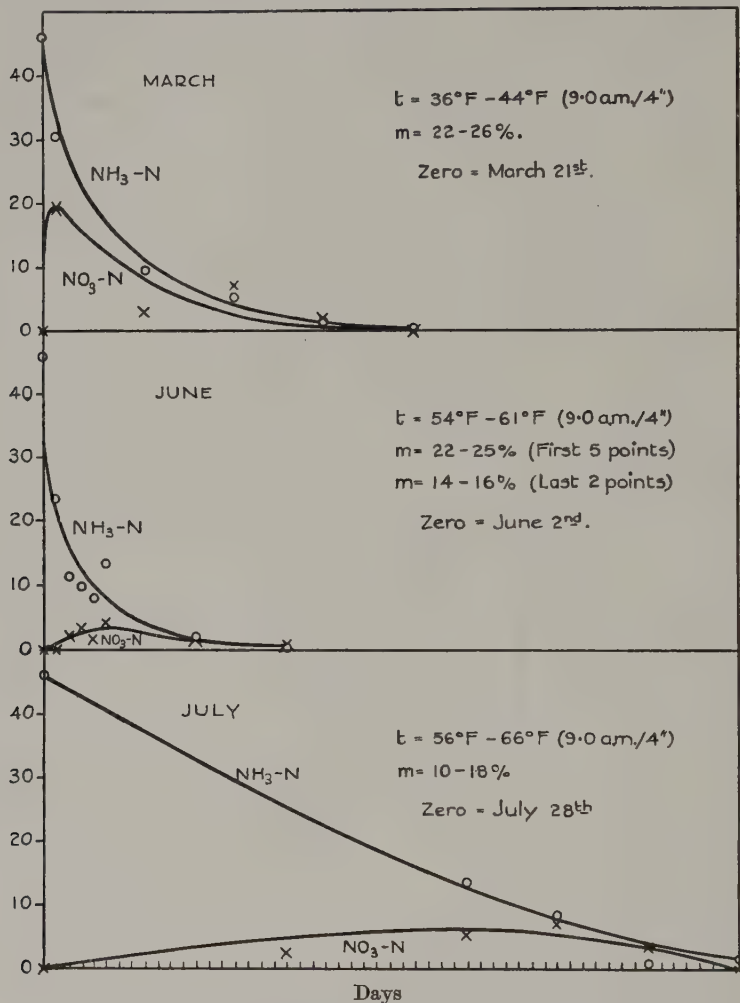


Fig. 2. Graph showing disappearance of $\text{NH}_3\text{-N}$ and accumulation of $\text{NO}_3\text{-N}$ at different times in the season. Drownboy Field, 1933.

The second application of nitrogen in June did not produce such a marked effect—presumably because the grass was in more effective competition with the micro-organisms. The third application, which was made in relatively dry weather (July), produced hardly any increase at all in so far as the hiatus in the data permits any conclusions to be drawn. Over the whole period there is a marked regression in numbers

on the treated plots relative to the non-treated plots, which is undoubtedly due partly to the acidifying nature of the fertiliser used (sulphate of ammonia) and partly to the fact that the soil moisture was noticeably lower on the N-treated plots, a fact arising from the larger amount of water transpired by the larger amount of grass on the treated plots.

The numbers of actinomyces showed a doubtful tendency to increase on the un-irrigated soil, but showed no change on the irrigated soil. The fungi similarly do not appear to be influenced.

In Fig. 2 I have shown the curves for the disappearance of ammonia and accumulation of nitrate-nitrogen in the experiment to which I have just referred. These figures were obtained from an analysis of the soil extract prepared by the method suggested by C. Olsen, and represent differences between the figures for the treated and untreated plots. Taking differences like this has the effect of smoothing out the natural fluctuations which are common to both plots. The upper portion of the graph refers to the March application of sulphate of ammonia. You notice that the disappearance of the sulphate of ammonia is accompanied by an accumulation of nitrate, which reaches a maximum very shortly after the application of the ammonia. The grass was just beginning to grow at this time, and the fall in the nitrate curve is very largely a reflection of the increasing activity of the grass. No trace of the added nitrogen, either in the form of ammonia or nitrate, remained after 4 weeks. Note the moisture and temperature level.

The middle portion of the graph refers to the June application which, as you see, disappeared in a much shorter time. In fact, over half the added nitrogen had disappeared within 2 days. Growth conditions were optimum at this time (moisture 23-25 per cent. and temperature about 60° F.). The uptake of nitrogen by the plant was probably more rapid at this time than at any other. The lower portion of the graph shows the state of affairs when the fertiliser was applied in the relatively dry conditions of July. Although samples were not taken at the actual time, it may be inferred from samples examined 2 weeks previously and 2 weeks subsequently and from a knowledge of weather conditions during the period, that the moisture content of the soil at the time of application must have been in the neighbourhood of 10 per cent.; and similarly it may be inferred that there were considerably fewer soil organisms at this time than there had been in the spring. At all events, the rate of disappearance of the ammonia was very considerably reduced at this time, undoubtedly owing to the limiting effect of moisture. It is not until nearly 2 months after application that the fertiliser can be said to have disappeared.

The disappearance of the ammonia is, of course, mainly due to its oxidation to nitrate and assimilation by the grass and those organisms in competition with the grass, but it is certain that not all the ammonia is thus oxidised. There is definite evidence that some is taken up by the grass as ammonia.

Table I.

Showing direct uptake of NH₃-N by grass. Drownboy Field, 1933.

Days	1	2	3	9	24
In control soil (p.p.m. dry soil)	6.7	8.2	7.6	11.1	6.8
In N-treated soil (p.p.m. dry soil)	5.6	34.9	43.4	14.8	10.0
In control grass (p.p.m. dry matter)	480	380	450	500	390
In N-treated grass (p.p.m. dry matter)	390	920	640	830	320
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In Table I are shown some figures obtained in an experiment conducted in 1933, in which the amount of $\text{NH}_3\text{-N}$ in the grass and in the soil was determined before and at various intervals after addition of sulphate of ammonia. From these figures there would appear to be no doubt whatever that the ammonia is taken up directly by the grass. The sulphate of ammonia was applied 2 hours before sampling on the second day, adequate steps being taken to prevent any sulphate of ammonia adhering to the foliage.

Fig. 3 shows some results obtained in an experiment conducted last season, where, amongst other things, the nitrate and ammonia contents were determined at frequent

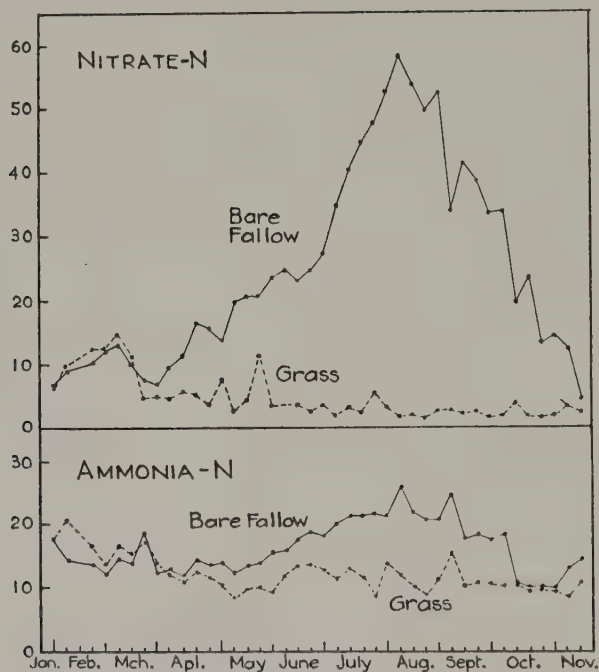


Fig. 3. Graph showing seasonal variations of $\text{NO}_3\text{-N}$ and $\text{NH}_3\text{-N}$ in adjacent plots of bare fallow and grassland soil. Old Meadow Field, 1934.

intervals throughout the season in soil upon which grass was growing, and in adjacent or rather intermixed plots which were kept free from grass and weeds. The nitrate-N you will see, commenced to accumulate on both plots during February, which was an unusually dry month. Heavy rains in early March, however, caused a loss of nitrate from both plots. At this time the grass began to grow, and you will notice how the nitrate disappeared more quickly on the plots where grass was present. From the end of March nitrate began to accumulate rapidly, on the fallow plots, until by August it had reached nearly 60 p.p.m. From then on it began to disappear, until by the middle of November there was no appreciable difference between the nitrate contents of the two plots. During all this time on the grass plots the nitrate never amounted to more than

a few parts per million. On the lower half of the graph are shown the curves for ammonia-N. Here again the ammonia level was considerably lower on the grass plots than on the fallow plots, a fact which supports the view that the ammonia was being assimilated by the grass, although this must be discounted slightly by the possibility

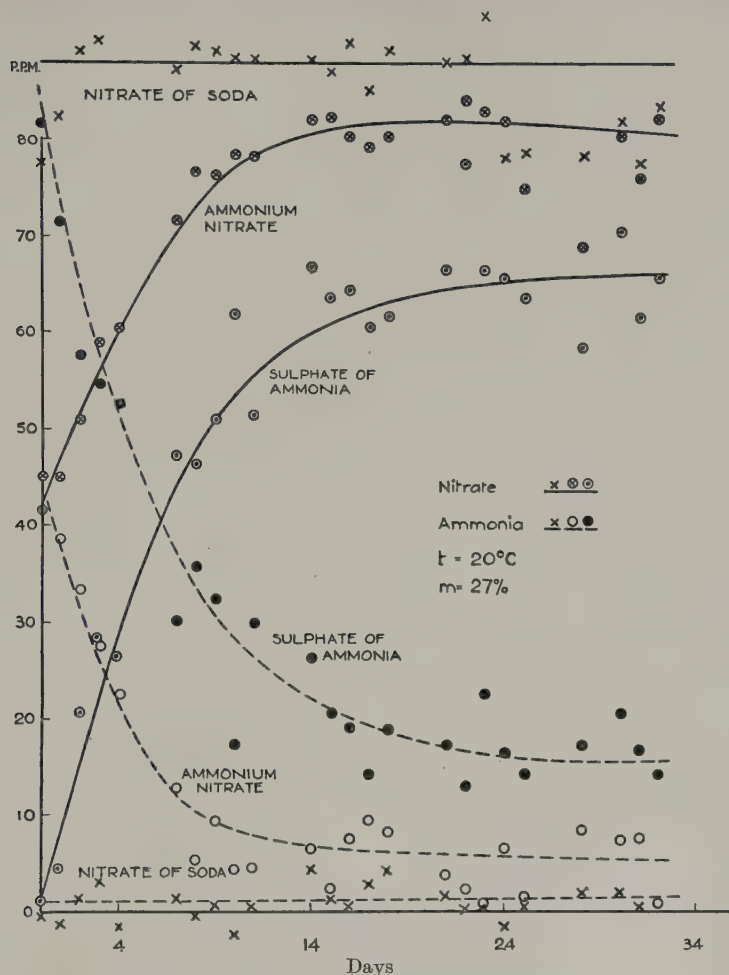


Fig. 4. Incubation experiment, 1934. Nitrification of S/A and A/N in grassland soil. Soil from Old Meadow Field.

that either (1) the slightly lower moisture content of the soil under grass may have depressed its ammonifying power somewhat, or (2) if this effect is negligible, nitrification may have been more intense under grass than under fallow, other things being equal, although, of course, the conditions for accumulation are much more favourable in fallow soil during summer. You will notice that in February the ammonia level is

higher on the plots sown with grass: this may have arisen from the decomposition of the chaff in the seed sown, and also from dead seeds and seedlings. Calculation based on the known N content of seed and chaff and on the percentage of chaff in the seed, showed that between 1-2 p.p.m. $\text{NH}_3\text{-N}$ could have arisen from the chaff alone. It was noticed also that numerous seedlings were killed off by frost. The disappearance of this excess of ammonia during February is accompanied by an appearance of nitrate. Nitrification was thus going on at a temperature definitely too low for the growth of the grass.

The next step in this investigation was obviously to obtain some idea of the distribution of nitrogen during the process of oxidation from ammonia to nitrate. For this purpose soil was taken from the plots used in the 1933 experiment, sieved, and filled into a large number of press-cap jars; between 200 and 300 were used in all. Each jar contained a known weight of moist soil of known moisture content. The jars were divided into 4 series. The first series served as a control. To the second series a known amount of sulphate of ammonia was added, 88 p.p.m. $\text{NH}_3\text{-N}$; to the third was added the same amount of nitrogen, but in the form of ammonium nitrate ($\frac{1}{2} \text{NH}_3$; $\frac{1}{2} \text{NO}_3$); and to the fourth series nitrate of soda was added, also at the rate of 88 p.p.m. $\text{NH}_3\text{-N}$. This amount of N corresponded to an application in the field of 3 cwt./acre of sulphate of ammonia. Every weekday during the following 5 weeks two jars were taken from each series and analysed for ammonia nitrate, nitrite and albuminoid N. The jars were aerated at intervals, and the moisture content and temperature were sensibly constant over the period of the experiment.

Fig. 4 merely shows the curves for the disappearance of ammonia and accumulation of nitrate. I should mention that these figures again represent differences from the control. This had to be done because the control jars showed a rapid increase in nitrate content, and it was necessary to distinguish between the nitrate arising naturally from the soil and that arising from the added ammonia. The figures are of little interest here, except in showing that the soil from this experiment possessed a strong nitrifying power.

From the known amount of nitrogen added and the sum of the nitrate and ammonia in excess of any *natural* increase or decrease, it is possible to express that found as a percentage of that added. If the amount recovered falls short of the theoretical amount, then either some of the N is existing in a form not estimated, or some has left the system as a gas. In Fig. 5 are shown the recoveries obtained.

The first thing to notice is that the mean recovery in terms of $\text{NH}_3\text{-N}$ and $\text{NO}_3\text{-N}$ for the first week (each point being a mean of two determinations) was only 91 per cent. of the nitrogen originally added in the ammoniacal form. For the second week the recovery improved to 92.6 per cent. In the third week it was 94.8 per cent., and in the fourth week it was 99 per cent. In the second case, where the nitrogen was added as ammonium nitrate, a similar though not so regular improvement is also noticed. Whereas the mean recovery for the first week was 91 per cent. where the nitrogen was all in the ammoniacal form, here with half ammonia and half nitrate the recovery is half-way between 91 per cent. and 100 per cent. Finally, where the nitrogen originally added was all in the nitrate form, it is interesting to note that about 10 per cent. of the nitrogen added was not recoverable within the first two days. During the subsequent two or three weeks the recovery averaged 100 per cent., but the recoveries were not so good during the last week of the experiment. No nitrite-N was found at any time.

It was thought that a perhaps better recovery figure could be obtained by taking into account that fraction of the nitrogen which is obtained from the extract as ammonia by boiling with alkaline permanganate—a fraction which is usually referred

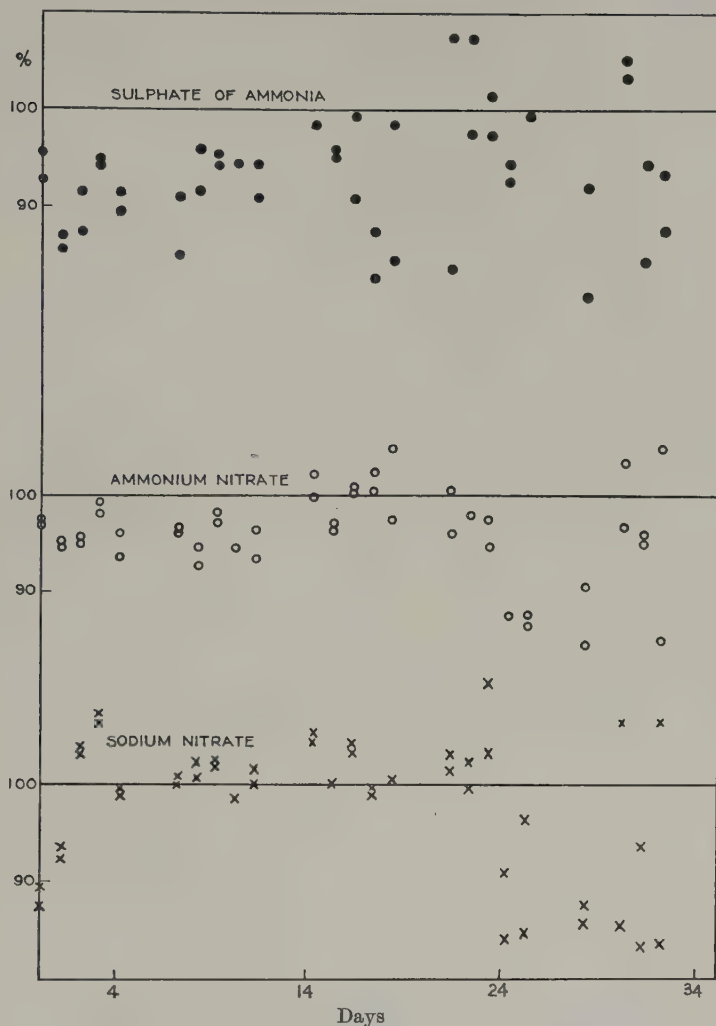


Fig. 5. Incubation experiment, 1934. Per cent. recoveries ($\text{NH}_3 + \text{NO}_3$).

to as albuminoid ammonia. There was justification for this view, since in the experiment referred to earlier it was noticed that the albuminoid-ammonia figures were always higher immediately following an application of ammonium nitrate. The graph in Fig. 6 shows this quite clearly.

However, these hopes were not realised. The recoveries calculated on the basis of $\text{NH}_3\text{-N} + \text{NO}_3\text{-N} + \text{extractable albuminoid ammonia}$ are shown in Fig. 7, and you will see that they are no better than those calculated on a basis of $\text{NH}_3\text{-N}$ and $\text{NO}_3\text{-N}$ only. The points have been joined up merely to enable the two sets of data to be distinguished.

The curves show that during the first week 8-9 per cent. of the ammonia-N originally added as sulphate of ammonia is not recovered by extracting with Olsen's extraction medium. A similar fraction of the ammonia-N originally added as ammonium nitrate is also not recovered.

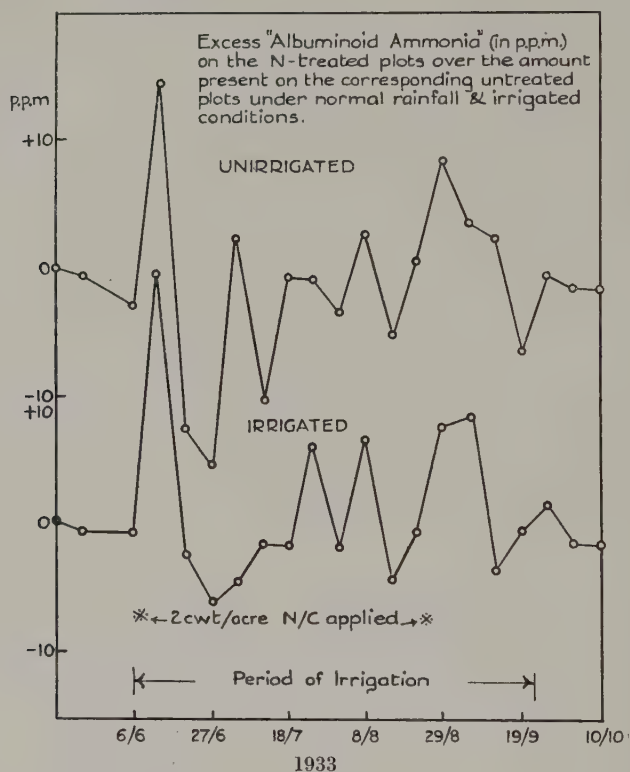


Fig. 6.

This apparent loss can arise from three causes:

- (1) Incomplete extraction of the absorbed $\text{NH}_3\text{-N}$, in which case, as nitrification proceeds, better recovery should be obtained.
- (2) Uptake by micro-organisms and conversion into some compound of nitrogen which is not usually determined.
- (3) A portion of the original $\text{NH}_3\text{-N}$ existing in a partially oxidised form in which it is not estimated, *i.e.* a form other than $\text{NH}_3\text{-N}$, $\text{NO}_3\text{-N}$ or $\text{NO}_2\text{-N}$, in which case also an increased recovery should be obtained as nitrification proceeds.

A loss might also be imagined to arise by interaction of HNO_2 and primary amines or ammonia, or by some other decomposition process involving elimination of gaseous N, but since the bulk of the deficiency is, as we see, ultimately made up, this alternative would appear to be ruled out to a very large extent, at least under the conditions of the experiment.

In Olsen's original work he took great care to test a large variety of soils in order to show that the method of extraction he advocated was of universal application. The soils

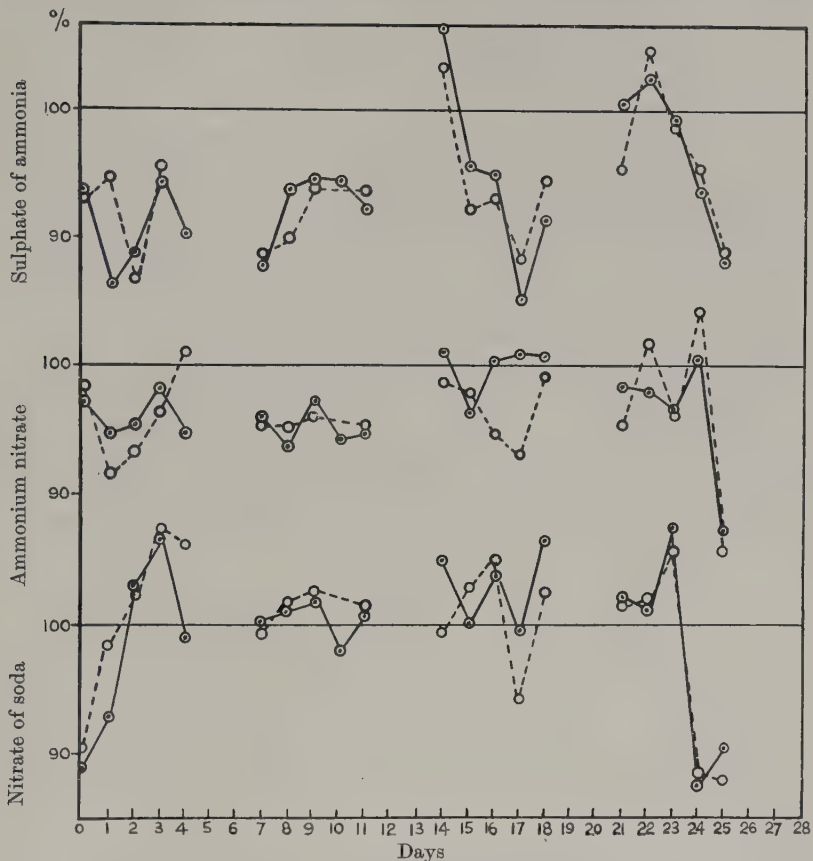


Fig. 7. Mean (2) daily per cent. recoveries. \odot — \odot Recoveries calculated on inorg.-N.
 \bigcirc — \bigcirc Recoveries calculated on inorg.-N + albuminoid-NH₃.

he used ranged from a heavy calcareous clay soil, rich in humus, to a partly decomposed high moor peat, and in all cases he was able to recover 100 per cent. of the ammonia which he added when he extracted *immediately*. A few examples are given in Table 2.

From these figures there would appear to be little doubt that the Olsen method of soil extraction is 100 per cent. efficient, and that the ammonia added to the soil can be completely recovered on immediate extraction by this method.

Table II.

Showing amounts of ammonia extracted from various soils (C. Olsen).

	Known to be present, p.p.m.	Found p.p.m.
Heavy calcareous clay rich in humus, pH 7.6	163	162.6
Heavy clay soil poor in humus, pH 5.4	163.1	163.4
Slightly calcareous sandy soil rich in humus, pH 7.5	182.5	181.3
Partly decomposed high moor peat, pH 3.6	1089	1092

So that we are apparently left with the fact that, of the original nitrogen added, a portion is not recoverable for some time, either as ammonia-N, nitrite-N, nitrate-N or albuminoid-N. The balance of evidence appears to favour the view that this missing nitrogen represents that utilised in producing a temporary increase in the numbers of certain organisms, most likely those concerned directly in nitrification, although the possibility that a small margin may exist as hyponitrite is certainly not excluded, as you will have gathered from Dr Corbet's paper. Indeed it is gratifying to note that these results receive considerable support from his work.

It might be mentioned in conclusion that hyponitrite would definitely escape detection by the methods of soil extraction and analysis usually employed.

Work is now in progress at Jealott's Hill in which it is hoped to find a satisfactory method of extracting and estimating any hyponitrite which may normally occur in soils, and if such a method can be evolved, we shall be able to obtain a much clearer idea of the conditions favouring its formation and accumulation.

REVIEWS

An Introduction to Plant Biochemistry. By CATHERINE CASSELS STEELE. Pp. viii + 356, figs. 12, tables 6. London: G. Bell and Sons, Ltd. 1934. Price 15s.

Biochemistry is a large and ill-defined territory merging into organic chemistry on the one side and into physiology on the other. At need, botanists remember Czapek's ponderous tomes, but leave them on the shelf; Plimmer's volume which is convenient but, for the botanist, unsatisfactory and often exasperating; books by Onslow and by Haas and Hill which they find useful but not sufficiently physiological; various "Plant Physiologies" which are not sufficiently biochemical. There has been room for a smallish self-contained book, suitable and convenient for student use, which would give the elements of plant biochemistry in relation to plant physiological processes. Dr Steele has, to a large extent, met this need and her book, which is not a work on biochemistry at large but definitely on plant physiological biochemistry, is an excellent production.

From the beginning the subject is developed logically according to modern theories of organic structure and, although here and there, as, for example, in grouping the aldehydes with the carbohydrates rather than between the alcohols and acids, the author modifies the usual sequence of compounds, such rearrangements have the advantage of juxtaposing substances intimately related in the metabolic processes of the higher plants. The biochemistry of lower plant forms is used for purposes of comparison and illustration but is not dealt with in detail.

The book is divided into seven sections. Part I is introductory and contains two chapters dealing respectively with the chemical composition of plants, and the colloidal state. Part II contains four chapters dealing with the alcohols, the fatty acids and the fats and oils. Part III contains five chapters dealing with the aldehydes, ketones and carbohydrates. Part IV consists of one chapter on plant acids. Part V contains four chapters dealing with proteins and related compounds. Part VI contains five chapters dealing with cyclic compounds, including aromatic compounds, plant pigments, alkaloids and essential oils. Part VII contains six chapters dealing with plant metabolism, including enzymes, photosynthesis and the carbon metabolism of plants, nitrogen metabolism, respiration, the chemistry of plant growth, and maturation, fruit ripening and storage and the chemical effects of cold and frost on plants. The volume concludes with a short bibliography, an index of over 500 botanical names and a good general index.

The book is up to date, is simply and clearly written and seems to me a wholly admirable introduction to plant biochemistry, Parts VI and VII being particularly good. It is an elaboration of a series of about thirty lectures suitable for second or third year students in botany, and the accompanying experiments, which are inserted appropriately throughout the book, form a parallel course of practical instruction. The experiments are all such as could be carried out in a plant physiological laboratory with ordinary equipment and form a well-arranged course. The cost of the book seems a little excessive.

WILLIAM B. BRIERLEY.

1. *Virus Diseases of Plants: A Bibliography.* By D. ATANASOFF. Pp. iv + 219. Sofia: Houdojnik Printing Co. 1934. Price \$3.
2. *Partial Bibliography of Virus Diseases of Plants.* By J. I. OTERO and M. T. COOK. Pp. 401. (*Journal of Agriculture of the University of Puerto Rico*, xviii, 1-2, January-April, 1934.) Agricultural Experiment Station, Rio Piedras, P.R. 1934.

References to the virus diseases of plants are scattered through the botanical, horticultural and agricultural literature of the last 200 years but, during this century

and especially since the European war, they have multiplied almost out of control. Many of them are collected and classified in K. M. Smith's valuable book but this omits the older papers and many others which have appeared in out of the way journals. The authors of the present works have performed the very useful but somewhat thankless task of searching the world's literature for plant virus references and collecting them in easily available form.

Atanasoff classifies his bibliography into: I, Works treating fundamental problems of virus diseases; II, Works treating different virus diseases of various plants; III, Works treating specific virus diseases of plants arranged by hosts in families; and IV, Works treating insect vectors of virus diseases. Section IV includes only thirty-four titles since many of these papers are cited in section I. Where, in section III, a family of plants is large and important the references are further classified. In the Solanaceae, for example, the sub-divisions are as follows: (a) Virus diseases of various plants; (b) Pepper virus diseases; (c) Potato various virus diseases up to 1916; (d) Potato various virus diseases since 1916; (e) Potato calico or aucuba mosaic; (f) Potato leaf burn; (g) Potato leaf roll; (h) Potato mosaic; (i) Potato net necrosis; (j) Potato psyllid yellows; (k) Potato spindle tuber; (l) Potato crinkle, curly dwarf, yellow dwarf, rugose mosaic and streak; (m) Potato top necrosis; (n) Potato witches' broom, giant hill, and bolting; (o) Tobacco various virus diseases; (p) Tobacco crinkle, curly dwarf, kroepoek, witches' broom; (q) Tobacco mosaic; (r) Tobacco ring-spot; (s) Tomato various virus diseases; (t) Tomato aucuba or yellow mosaic; (u) Tomato mosaic; (v) Tomato spotted wilt; (w) Tomato streak, stripe, and winter blight; (x) Tomato western yellows. The citations, which number over 3700, are given in full, pagination usually being included; and in many cases reference is also made to abstracts in "referate" or the various abstracting and review journals. There are good author and host indices which make it simple to track down any particular citation in the body of the work.

The book by Otero and Cook contains 2337 references arranged alphabetically by author and chronologically under the name of each author. The citations are given in full with, in most cases, an English translation of the original title, and reference is also frequently made to abstracts in review journals. The individual citations are more useful than those given by Atanasoff since a brief abstract of the paper is usually appended. On the other hand the bibliography is not so complete and the book contains perhaps more misprints although, as both works sin rather badly in this respect, neither is sufficiently reliable to be used as a source of exact citation in the absence of the original paper.

Both works include 1933 investigations, and as the authors hope to publish supplements keeping their bibliographies up to date, they request that reprints of virus papers be sent to them. It is to be hoped that workers will comply with this request, since a complete and reliable bibliography of plant virus diseases would be a valuable tool. In such supplements the authors would be well advised to adopt the standardised abbreviations of journal titles given in the Oxford Press *World List of Scientific Periodicals*, 1934. The Bulgarian work is better on European and North American references and the Porto Rican work on Spanish-American references, and it is a great pity that both bibliographies are not combined in one volume.

WILLIAM B. BRIERLEY.

Investigations on Barley. By Sir E. J. RUSSELL and L. R. BISHOP.
Institute of Brewing, Brewers' Hall, Addle Street, London, E.C. 2.
1933.

In 1922 the Barley Committee of the Institute of Brewing Research Scheme initiated work on the influence of environmental conditions on the yield and quality of barley, the possibility of developing improved malting barleys and the relation of chemical composition of barley to malting and brewing value. The research was

centred in the Rothamsted Experimental Station and numerous scientific papers and reports giving an account of the progress of the investigations have appeared. The results of the ten years' work have now been collected and synthesised in the present publication which thus brings up to date the valuable report issued by Hulton in 1922.

Comparison of the two reports gives one a very good idea of the new biochemical, physiological and statistical methods of attack which have been developed during the interim period. In spite of all the advance, however, the authors must still admit that "We are not yet in a position to give a full definition of what constitutes brewing quality or to say how, if at all, it can be measured." Although certain problems have been definitely solved much of the work during the decade had to be expended on clearing the site and building foundations. Perhaps the most important scientific results of the investigation are the demonstration of the distinctiveness of the nitrogen patterns of barley varieties, the clearing up of the "nitrogen controversy", and the tracing out of the effect of weather conditions on the nitrogen content of barley. An outcome of considerable practical importance is that high yielding varieties contain lower percentages of nitrogen than low yielding varieties and that no indication was found of any possibility of producing high yielding varieties of high nitrogen content.

The second half of the book consists of tabulations of primary data from the barley experiments conducted by the Institute of Brewing during 1922-31 which have been drawn up by Messrs H. M. Lancaster, H. Lloyd Hind and F. E. Day. There are author and subject indices.

The book is a résumé in plain intelligible language of a very great amount of highly technical research carried out in the laboratory and field. From many points of view the research scheme, the way in which the investigations were organised and carried out, the maintenance throughout of a nice balance of theory and application, the frequent publication of results and a final synthesis such as the present publication seem to me to form a model which many industries might well follow when confronted with urgent and difficult problems. Both science and practice stand to gain by such a liaison.

WILLIAM B. BRIERLEY.

The Gramineae, a Study of Cereal, Bamboo, and Grass. By AGNES ARBER.

Roy. 8vo. Pp. xvii + 480, frontispiece and 212 text-figs. Cambridge: University Press. 1934. Price 30s.

The study of the Gramineae is of first importance both theoretically and practically. Systematically, the Order presents innumerable problems which challenge the basic concepts of orderly arrangement and descriptive classification in plants. Morphologically and anatomically, the Order exhibits, within a definite theme, variations of such number and magnitude as often to demand a larger and different keyboard from that used in our structural and phylogenetic improvisations. Ecologically, the gregariousness of the Gramineae is almost virgin scientific territory and presents fascinating problems whose solution would throw a flood of light on the social relationships, distribution and dispersion of plants. Physiologically, grasses have been little explored and yet the individual genotype can be so purified and standardised that, wheat physiology, for example, might almost play the function in general plant physiology that the hydrogen atom plays in general chemistry. Genetically, the Order exemplifies almost the whole range of phenomena known in plants. Pathologically, the Gramineae is the host Order *par excellence* of the rusts and smuts and presents more fundamental problems of disease than probably any other Order. Agriculturally, the Gramineae are more widely cultivated and more intensively studied than any other plants and, economically, the Order contains staple food plants on which man's very life and society depend.

The number of books and memoirs on the Gramineae is legion and to select any one as most important or most interesting is to invite obloquy. Yet, to my mind, Mrs Arber's volume stands in a class by itself both for scientific value and interest. It is

written out of a great knowledge and experience of the subject; it shows that masterly handling of data and spontaneity of treatment which only many years of first hand experience can give, and it possesses a philosophic comprehension and a processional ordering which makes it almost of epic quality. Much of the actual writing is, of course, precise description of botanical form and structure, straight hard scientific writing of a high level, but permeating the whole book is a certain fragrance, a controlled discursiveness and wide-ranging quality that one always associates with the great herbalists of a bygone age. It is a style of writing almost unique in the professionalised botany of to-day and it is a delight to read. It contains far more of the author's personality than any other larger technical book on botany I know, and when one has finished it, like Oliver, one asks for more.

The subtitle of the volume, "A Study of Cereal, Bamboo, and Grass", indicates the author's selection in the vast field before her. Within these narrowed limits she has constructed her work about the theme of the pattern and rhythm underlying the complex of plant types called the Gramineae. The book contains little of ecology or pure systematics and practically nothing of plant pathology or physiology but it contains much historical lore, much solid detail of morphology and anatomy and, suffusing all, a wide knowledge and appreciation of the more general biological aspects of her subject, and a deep feeling for plants as living things.

The chapter headings give little indication of the way the author treats her subject or of the wealth of information the book contains but, such as they are, they are given: I, Cereals of the Old World; II, Cereals of the East and of the New World: General Conclusions; III, Pasture, Sugar, and Scent; IV, Bamboo: Vegetative Phase; V, Bamboo: Tree Habit; VI, Bamboo: Reproductive Phase; VII, Bamboo: Spikelet and Fruit; VIII, The Reproductive Shoot in Grasses: Structure and Anthesis; IX, The Reproductive Shoot in Grasses: Compression and Sterilisation; X, Individuality and Life-phases in Bamboo and Grass; XI, The Grass Embryo and Seedling; XII, The Vegetative Phase in Grasses: Root and Shoot; XIII, The Vegetative Phase in Grasses: the Leaf; XIV, The Gramineae and the Study of Morphological Categories; XV, The Distribution and Dispersal of Grasses; XVI, Maize and Townsend's Cord-grass: two Putative Hybrids; XVII, Pattern and Rhythm in the Gramineae.

The book concludes with a short "Taxonomic Table", a "Bibliography" and a good "Index". The bibliography is limited to memoirs actually cited in the text, but even so runs to forty-four pages.

It is quite impossible in any notice of reasonable length even to indicate the wealth of knowledge and experience the volume contains. The greater part of the morphological treatment is based upon the author's own researches which have appeared year after year in the *Annals of Botany* and other journals since 1917 and most of the illustrations derive from this source. Mrs Arber's exquisite and informative line drawings and slightly back-hand lettering are familiar to all botanists and, in this book, show the same over-reduction and sardine-like packing that have tired readers' eyes in the past. Mrs Arber's appreciation of the folk-lore aspects of plants and their history in cultivation is strongly marked throughout the book and has led her to include a large number of delightful illustrations culled from herbals and other early works. The frontispiece is a beautiful reproduction of Albrecht Dürer's water colour "Das Grosse Rasenstück".

The volume is not only a mine of information but is extraordinarily suggestive, propounding quite as many questions as it answers. The suffusion of the work by Mrs Arber's personality, and the frequent blunt statement of her personal opinion on matters severely botanical and on others having wider controversial implication makes the volume very stimulating. This is especially so when the author's opinions are directly opposed to those of the reader, which must quite frequently be the case.

Thinking back over the book, the main ideas that seem to stand out in Mrs Arber's work are the variability in structure and mode of life within one primary pattern, the conception of the tree habit as an expression of racial senile degeneration, the fundamental importance of parallelism and repetition in the Order, the primacy and equivalence of shoot and root categories and the lack of fundamental distinction between stem and leaf, the high significance of node and internode and the importance

of compression in the bud stages tending to reduction in reproductive shoots, and a general trend towards advancing sterility.

Mrs Arber at first considered that in an entirely logical treatment of the Gramineae "their relation to man would be ignored, or consigned to appendices". Yet this is a human logic devised by man and applied by man to organisms many of which have been originated by man and bred and cultivated by man and exist solely for human purposes, and are now described in a book presumably to be read by human beings. Inevitably Miss Arber could not endow such dead bones—she describes them as "a logical skeleton"—with vitality. She therefore turned from necrology to biology and her book "begins with the study of the grasses in relation to man, and the more strictly botanical aspect is treated as developing out of the humanistic". She has clothed her "logical skeleton" with human flesh and blood, and it breathes the breath of life.

WILLIAM B. BRIERLEY.

A History of Embryology. By JOSEPH NEEDHAM. Pp. xviii+274, text-figs. 40, plates XVI, charts III. Cambridge: University Press. 1934. Price 15s.

The name "Needham" has an honoured place in the history of biology during the seventeenth and eighteenth centuries and, in this twentieth century, yet another Needham is making for himself a place in future histories. *Science, Religion and Reality*, 1925; *The Sceptical Biologist*, 1929; *Chemical Embryology*, 1931; *A History of Embryology*, 1934: there may be other volumes for all I know, but these happen to have come my way and all are works of which any man might well be proud. The present volume follows closely the historical essay on "The Origins of Chemical Embryology" from the earliest times to 1814 in vol. I of the author's *Chemical Embryology*, but the text has been revised throughout and expanded by the addition of interesting footnotes. I described the original essay (see *Ann. appl. Biol.* XIX, No. 2, May 1932) as "a finely written and very scholarly history of the subject" and the present work is even better. A notice of such a book as this has, perhaps, no place in a scientific journal devoted to Applied Biology, but I have so enjoyed reading it that I cannot forbear to express my appreciation. The work is delightfully written and beautifully illustrated and it holds one's interest from beginning to end. The last chapter, some ten pages of general conclusion, is one of the best things I have read for some time. There is a splendid Bibliography which, however, omits Senn's recent *Die Entwicklung der Biologischen Forschungsmethode in der Antike und ihre Grundsätzliche Förderung durch Theophrast von Eresos*.

WILLIAM B. BRIERLEY.

Report on Fungus, Bacterial and other Diseases of Crops in England and Wales, 1928-32. Ministry of Agriculture and Fisheries Bulletin No. 79. H.M. Stationery Office. 1934. Price 2s. net.

Of the many valuable publications of the Ministry of Agriculture and Fisheries one which pathologists eagerly await is the report of the survey of fungus and other diseases of crops. The present issue, covering the years 1928-32, is constructed on very much the same lines as in previous years but contains a little more introductory matter, useful notes being included on progress in control measures and on scheduled plant parasites. The individual diseases also receive fuller treatment and, as much attention has been given to the collation of literature references, the bulletin serves not only as an account of the incidence of plant diseases during particular years but as an excellent

handbook to recent pathological work. An increased number of diseases is recorded, the present bulletin indexing 396 fungus and bacterial parasites and 54 non-parasitic and virus diseases as against 301 and 25 respectively in the issue for 1925-7. The records of special interest include certain virus diseases and 33 pathogenic fungi and bacteria which are either new to science or previously unrecorded in Britain.

This survey work, carried out under the direction of Dr G. H. Pethybridge with the able assistance of Mr W. C. Moore and Dr A. Smith, and over eighty collaborators, is a vital part of the plant disease work in this country and with the passage of years the data become more valuable owing to the increased basis of comparison. The bulletin itself with its authoritative treatment and standardised nomenclature, the admirable arrangement of its contents, its illustrations and adequate indexes is a publication of first class importance serving both as a contemporary historical document and as a most useful *vade-mecum*. Published at the low price of two shillings it should be a personal possession of all interested in the growing of healthy agricultural and horticultural plants.

WILLIAM B. BRIERLEY.

The Life Forms of Plants and Statistical Plant Geography, being the collected papers of C. Raunkiaer. Pp. xvi + 672, figs. 189. Oxford: at the Clarendon Press. 1934. Price 35s.

Raunkiaer's work and, perhaps especially, his system of life forms, is of fundamental importance in vegetation study, but it has not yet exercised its full influence owing to the fact that most of it was published in Danish periodicals not easily available. The translation of these seventeen papers into most readable English and their inclusion within one volume is, therefore, a noteworthy event in the history of the science. Raunkiaer's studies covered a very wide field ranging from exact morphological investigation to descriptive and geographical plant ecology, extensive quantitative and statistical analysis of plant formations and critical physiological research on plant environmental relations.

Ecology and agriculture are rarely classed together, yet crop culture is essentially the carrying out, under partially controlled conditions, of ecological experiments on economic plants. These experiments are performed by agricultural scientists for their own purposes, or by farmers and growers in the pious hope of a cash return, but the experiments themselves are a source of ecological data having primary value. A field of potatoes, a tea plantation, or a glasshouse tomato crop present just as many and just as fundamental ecological problems as a sand dune or a salt marsh, and have the inestimable advantage that we know more about the plants concerned and the conditions under which they are growing, and can not only control both plants and conditions but can investigate them more easily and repeat our experiments. The ecological investigation of a relict wheat field, a mixed pasture or a glasshouse crop has just as much pride of place as the ecological investigation of a sea shore or a woodland area and is just as likely, if not more likely, to give results of scientific value.

Many of Raunkiaer's ideas are directly applicable in the ecological study of crop plants growing in pastures and other agricultural and horticultural formations and, with the increasing attention being given by agricultural botanists to the ecological aspects of their problems, this Danish work merits the most serious consideration. The book itself is a joy to handle being well illustrated and beautifully printed and produced.

WILLIAM B. BRIERLEY.

Plant Chimaeras and Graft Hybrids. By W. NELSON JONES. (Methuen's Monographs on Biological Subjects.) Pp. viii + 136, figs. 22. London: Methuen and Co., Ltd. 1934. 3s. 6d.

Plant chimaeras and graft hybrids have long provided a source of very intriguing problems for botanists and horticulturists and their interest and usefulness are by no

means at an end. A good summary of the data treated from the special genetic viewpoint of vegetative segregation appeared by Chittenden in *Bibliographia Genetica*, 1927, and an admirable historical treatment of the "classical examples" was contributed by Weiss to *Biological Reviews*, 1930. The most comprehensive recent account of the subject is in Krenke's *Wundkompensation, Transplantation und Chimären bei Pflanzen*, 1933.

The present essay by Neilson Jones is not meant in any way to supersede the above, but the author critically surveys the field and gives a clear picture of the subject and of the several interpretations which have been put forward to explain the botanical problems involved. He does not attempt to provide a complete record of every plant described as a chimaera or graft hybrid, but illustrates the different types of structure and development by a study of the cases which have been most thoroughly investigated. The little book contains an astonishing number of well-organised data, but it is a pity that Asseyeva's work on the potato was omitted, since it throws a good deal of light on the situation in this plant. The author supports Baur's now generally accepted "chimaeral hypothesis", according to which the pattern found in the mature organ of a chimaeral structure is a development of the pattern already present at the growing point.

The book is the outcome of a series of intercollegiate lectures to advanced students of botany in London University, and it is an excellent statement of the present position which all botanical and horticultural students will find of value. It is well written and clearly illustrated and contains a useful selected bibliography. There is a spelling mistake on p. 113, and a wrong reference and four spelling mistakes in six lines on p. 121.

WILLIAM B. BRIERLEY.

The Genetics of Garden Plants. By M. B. CRANE and W. J. C. LAWRENCE.

Pp. xvi + 236, figs. 53 and 42 tables. London: Macmillan and Co., Ltd. 1934. 10s. 6d. net.

For some years there have been available good American text-books dealing with genetics in relation to agriculture although all of these sadly need bringing up to date. There has, however, been no volume covering the horticultural relations of the subject although the need for such a book has been very evident. Horticultural genetics is advancing so rapidly, is so complex and presents so few clear issues, that one must sympathise with geneticists in their hesitation to embark upon any text-book treatment of it. On the other hand, in many institutions courses on the subject are being given to students, and teachers look around hungrily for any book which they can recommend. Botanical and horticultural students do not want the principles of genetics illustrated, as they are in most text-books, by examples of *Drosophila*, mice, rabbits, cocks and hens and other stray flesh or fowl, they want to know how the principles are exemplified in plants. What is really needed is a volume on plant genetics and plant breeding; a synthesis of Sansome and Philip's *Recent Advances in Plant Genetics*, Hunter and Leake's *Recent Advances in Agricultural Plant Breeding*, Hayes and Garber's *Breeding Crop Plants*, and the present volume by Crane and Lawrence, the whole thing shortened and simplified and written in such a way that students of botany, agricultural botany and horticulture can all use it as a text-book. Until such a book becomes available we must be thankful for smaller mercies and the appearance of the present volume has been awaited with keen anticipation.

In their preface the authors state "Our plan, therefore, has been to describe principles as simply as the technicalities of the subject will allow; illustrating them with examples from a range of flowers, fruit and vegetables, and to give references to the original sources of information which may be of interest to the specialist or student. The book will, we hope, serve as an introduction to the science of genetics, and particularly in its application to horticulture."

Chapters I-III are introductory and deal respectively with the genetics of diploid plants, the cytology of diploid plants, and the cytology and genetics of polyploids. The next three chapters are complementary to the first three and exemplify genetic principles in flowering and ornamental plants, in vegetable and salad plants, and in large and small fruits, in each chapter a number of selected genera or species being discussed in turn. No hard or fast mode of presentation is adopted but, generally, a brief history of the plant in cultivation is followed by a consideration of its factorial composition, its breeding behaviour in regard to particular characters and, finally and very briefly, its cytology. The examples in each chapter are all carefully chosen to illustrate cytogenetic principles but the chapters would have been much more useful had more of the important horticultural plants found mention. Chapter VII entitled "Bud-sports, Variations and Fluctuations" is largely a discussion of the genetical aspects of plant chimaeras and might with value have been extended. The following two chapters contain a very clear résumé of the difficult and obscure problems of incompatibility and sterility in fruit trees, and are the best chapters in the book. The last chapter, the least satisfactory in the book, is concerned with the origin of new and improved forms. There is a useful glossary, a very incomplete bibliography and an adequate index.

"Colletotrichum" and "Synchytrium" are both wrongly spelled in text and index. On pp. 209-10 Baur's reference to "Peronospora" on the vine is taken from his J.R.H.S. Masters Lecture, and "Peronospora" should, of course, have been "Plasmopara": the retention of De Bary's old name is apt to lead to confusion.

The first three chapters of the book are the most concise introduction to technical genetics I know and must surely leave almost any grower or undergraduate student gasping. For anyone who has waded more leisurely through these troubled waters, and has become more or less familiar with them, it is a wholly admirable condensation but, as an introduction in itself, it seems to me to show a lack of understanding of what the subject looks like to an ordinary student or grower coming newly face to face with these problems. Advanced research workers are so steeped in their subject and so familiar with its jargon that they find it difficult to realise how strange and obscure it is to those without the pale. Anyone, however, who has really mastered these introductory chapters will be able to find his way through the rest of the book although, owing to the condensed and technical style of the writing adopted by the authors, his task will be far from easy. For the teacher the book is a first class guide through the jungle of horticultural genetics; for the ordinary student of botany, agricultural botany, and horticulture, it is, I think, far too condensed; for the grower it is too technical and insufficiently practical; for the post-graduate or research worker it is far too incomplete and selective. Still, it is the best thing to date, and we are duly grateful for such mercies as the John Innes Horticultural Institution doles out to us.

WILLIAM B. BRIERLEY.

REPORT OF THE COUNCIL OF THE ASSOCIATION OF APPLIED BIOLOGISTS FOR THE YEAR 1934

THE Association has met on six occasions during the year, including one field meeting and one excursion. The Annual Summer Meeting was held at the Wellcome Physiological Research Laboratories by kind invitation of the Director, Dr R. A. O'Brien, and the excursion was to the London School of Hygiene and Tropical Medicine. To both these Institutions the Association is indebted for their hospitality.

The attendance at ordinary meetings has been, on the average, 62 per meeting, of which number more than half have been members.

At a Special General Meeting held on February 16th, it was resolved that the name of the Association be changed to "The Association of Applied Biologists".

The Council have with regret to record the death of an Honorary Member, Prof. Neumann of Toulouse, and of a member of the Council, Dr R. C. Knight of East Malling Research Station.

Two new Honorary Members were elected, Prof. Ernst Gäumann and Mr B. P. Uvarov.

Eleven new Ordinary Members were elected during the year and two old members rejoined. Eight members resigned and the Association now numbers 280 Ordinary Members and 11 Honorary Members. Of the Ordinary Members 229 are resident in the British Isles and 51 in the Empire or foreign countries.

During the past year the Association has again enjoyed the privilege of holding its meetings in the Botanical Department of the Imperial College of Science and Technology, and the Council takes this opportunity of recording its grateful thanks, on behalf of the Association, to the College Authorities for this valued hospitality.

The following papers and discussions were brought before the Association during the year 1934.

- Feb. 16th.* Prof. W. B. BRIERLEY: Presidential Address, "Some Viewpoints of an Applied Biologist."
- Mar. 16th.* (1) Dr W. MALDWYN DAVIES: "The Sheep Blowfly Problem."
 (2) Mr E. R. SPEYER: "The Garden Symphid, *Scutigerella immaculata*, in Glasshouse soils."
 (3) Dr I. THOMAS: "Some Lesser-known Pests of Cereals with Observations on the Source of Infestation."
 (4) Mr A. S. BUCKHURST: "The Colorado Beetle."
- Oct. 12th.* (1) Dr A. C. THAYSEN: "The Origin of an Earthy, or Muddy, Taint in Fish."
 (2) Mr K. R. BUTLIN: "Enzyme Production: Note on the Oxygen Uptake of the Wash Liquid of Bacterial Suspensions."
 (3) Mr H. J. BUNKER: "The Distribution and Economic Importance of the Sulphate Reducing Bacteria."
 (4) Mr G. SAMUEL: "Impressions of a Plant Pathologist on a Visit to Java, Malaya and Ceylon."
 (5) Dr E. J. BUTLER: "Plant Pathological Problems in the Sudan."
- Nov. 9th.* (1) Prof. R. STEWART MACDOUGALL: "The Economic Importance of Arachnids."
 (2) Mr E. L. TAYLOR: "Applied Biology in the Control of Worm Diseases of Domestic Animals."

REPORT OF THE HON. TREASURER FOR THE YEAR ENDING DECEMBER 31, 1934

DURING the year ending December 31st, 1934, subscriptions and entrance fees (including arrears paid up) received from members amounted to £285. 18s. 0d. This is a decrease of over £20 from last year, and is less than the amount received in 1931 by over £50. Income from the sales of the current volume, from reprints and from contributions to the cost of papers in the *Annals of Applied Biology* amounts to £632. 7s. 2d., a decline of £92. The size of the volume of the *Annals* was less than in 1933 by 63 pages and the cost of producing it has fallen by £148 as compared with 1933.

Over the whole year there has been an excess of expenditure over income of £32. 11s. 9d. After all obligations have been met, the assets of the Association amount to £1084. 16s. 6d., of which £706. 5s. 0d. is represented by National Savings Certificates.

The financial position of the Association is therefore satisfactory at the present time. The decline in membership subscriptions as compared with last year and with 1931 is important, and it is most desirable that members shall pay their subscriptions promptly and take every opportunity to obtain new members.

J. HENDERSON SMITH,
Hon. Treasurer.

THE ASSOCIATION OF APPLIED BIOLOGISTS

Dr. *ANNALS OF APPLIED BIOLOGY* INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED DECEMBER 31st, 1934. Cr.

EXPENDITURE.		INCOME.	
	£ s. d.		£ s. d.
To Estimated value of Stock on January 1st, 1934	60 9 0	By Sales—Current Volume	511 16 0
To Cambridge University Press	933 0 6	By Sales—Back Volumes, Parts and Sets	13 16 11
To Copies bought in	16 10 0	By Sales of Reprints	100 4 3
		By Contributions towards cost of papers, etc.	12 10 0
		By Estimated Value of Stock on December 31st, 1934	85 18 6
		By Balance carried down	285 13 10
	<u>£1009 19 6</u>		<u>£1009 19 6</u>

Dr. *GENERAL INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED DECEMBER 31st, 1934.* Cr.

EXPENDITURE.		INCOME.	
	£ s. d.		£ s. d.
To <i>Annals of Applied Biology</i> , balance brought down	285 13 10	By Members' Subscriptions:	
To Printing and Stationery	17 18 6	Arrears	11 5 0
To Postages and Cheque Stamps	9 13 0	Entrance Fees	4 4 0
To Honorariums	10 10 0	Current	270 9 0
To Sundry Out-of-Pocket Expenses of Secretaries and Treasurer	10 1 9	By Interest on National Savings Certificates and Bank Deposit	285 18 0
To Audit Fee Reserve	4 4 0	By Balance, being Excess of Expenditure over Income for the year	26 15 0
	<u>£338 1 1</u>		<u>25 8 1</u>
			<u>£338 1 1</u>

BALANCE SHEET, DECEMBER 31st, 1934.

LIABILITIES AND SURPLUS.		ASSETS.	
	£ s. d.		£ s. d.
Sundry Creditors:		Cash:	
Cambridge University Press	323 3 4	At Bank on Current Account	256 11 7
Audit Fee Reserve	4 4 0	At Bank on Deposit Account	350 0 0
Sundry Expenses	14 15 9		606 11 7
Subscriptions and Entrance Fees paid in advance	342 3 1	Debtors for Subscriptions 2 years or less in arrear and considered good	41 5 0
Excess of Assets over Liabilities:	13 0 6	500 National Savings Certificates	706 5 0
As Balance Sheet of December 31st, 1933	1119 4 7	Stock of <i>Annals of Applied Biology</i> at estimated value	85 18 6
Less: Balance of Income and Expenditure Account for 1934	25 8 1		
	<u>£140 0 1</u>		<u>£1440 0 1</u>

J. HENDERSON SMITH, *Hon. Treasurer.*

We certify that the foregoing Accounts are properly drawn up in accordance with the books, vouchers and documents produced to us, and, in our opinion, the Balance Sheet exhibits a true and correct view of the state of the affairs of the Association.

H. J. COX & CO.
Incorporated Accountants. } *Auditors.*

Vol. XXII, No. 3

August 1935

THE ANNALS OF APPLIED BIOLOGY

EDITED FOR THE ASSOCIATION OF APPLIED BIOLOGISTS

BY

W. B. BRIERLEY

AND

C. T. GIMINGHAM

PUBLICATIONS COMMITTEE

W. BROWN

J. C. F. FRYER

A. C. THAYSEN

H. F. BARNES

J. HENDERSON SMITH



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Two important methods of determining and presenting ecological information are available: (a) the primarily biological method of recording the distribution of plant associations (cf. *Grassland Seeds* (7)), and (b) the mapping of the physical factors of the environment, a problem which appeals less to the biologist than to the geographer. The first method supplies data of much value relating to the prevailing biotic environment, and, inasmuch as they are reflected by the floristic distribution, to the physical factors also. A previous knowledge of the physical conditions is, however, essential for the correct interpretation of the biological observations. The two methods are therefore complementary to one another.

Some of the conditions imposed upon a growing crop are, within limits, controlled by man, e.g. cultural and manurial treatment, intensity of stocking, weed competition, etc., while others are beyond his control, e.g. temperature, rainfall, light, wind, etc. These latter factors being the natural attributes of an environment, frequently determine the extent to which an artificial environment can be created. They therefore form a useful basis for the initial charting of agricultural environmental regions. Such a recording of the comparatively stable *environmental coincidences* (Jarvis (8)) of agricultural regions, while paving the way for any detailed biological survey, would alone greatly facilitate the intelligent choice of crop variety trial areas, the scientific interpretation of trial results, and the logical introduction or local distribution of new crop plants.

II. PHYSICAL ENVIRONMENT OF THREE AGRICULTURAL REGIONS.

Region 1. An east coast region, comprising the counties of Berwickshire, East Lothian, Midlothian, Fife and Angus, and representing mainly the type of farming in which the growing of grain for sale plays an important part.

Region 2. A north-eastern region, comprising the counties of Aberdeen and Kincardine, in which cattle rearing and feeding predominate.

Region 3. A south-western region, comprising the counties of Renfrew, Ayr and Wigtown, representing dairy farming.

(i) *Topography and geology.*

Regions 1 and 3 consist largely of parts of the Midland Valley, a lowland generally of an undulating character formed on sedimentary rocks mostly of Carboniferous age in the south and Old Red Sandstone in the north. This is crossed by ranges of igneous hills, such as the Pentlands, Sidlaws and Renfrewshire Heights. Region 1 also includes part of the South-Eastern Highlands, a relatively high dissected plateau of

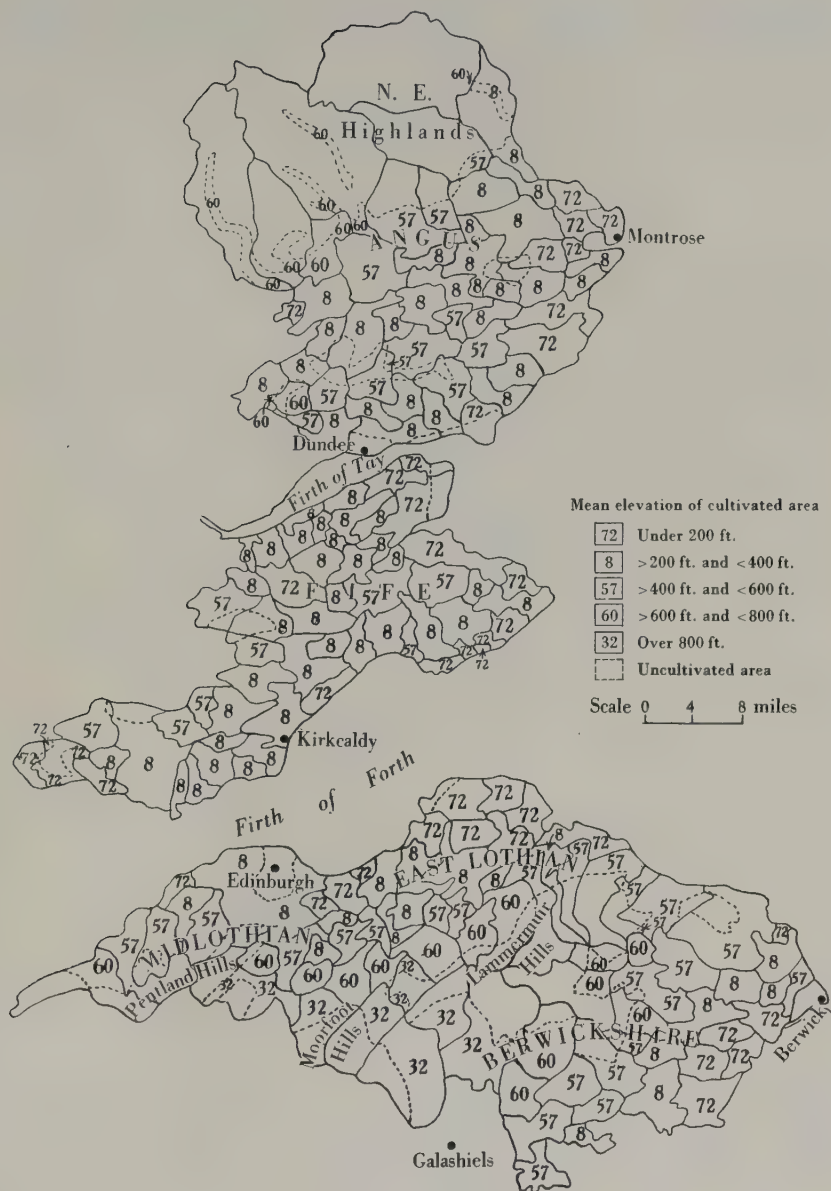


Fig. 1 a. Region 1.

metamorphic and granitic rocks, and the eastern end of the Southern Uplands, a lower dissected plateau of greywackes, grits and shales, which presents a steep face to the Lothians and sinks gradually southwards through a hilly zone of Old Red Sandstone to the drumlin-covered Carboniferous lowland of Berwickshire. Region 3 includes the western

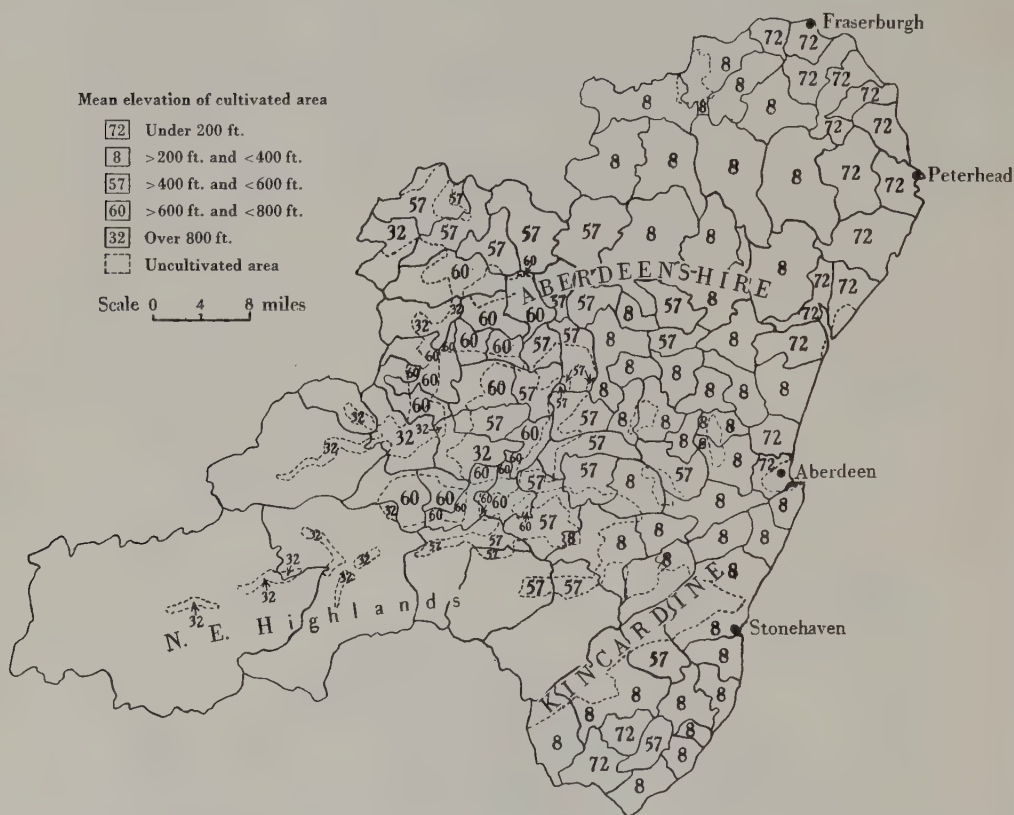


Fig. 1 b. Region 2.

end of the Southern Uplands and the Silurian lowlands of Wigtown which are in some parts hilly, in others flat and badly drained. Region 2 has a small part of the Midland Valley in south Kincardine and a large area of the Eastern Highlands in the west, but in the main consists of a flat to rolling lowland underlain for the most part by metamorphic and granitic rocks. Its soils are often poor and peaty, and large tracts were reclaimed from bog and moor during the last century.

The lower parts of all three regions are drift covered except on the sides of ravines, on some areas of igneous rock and in the eastern penin-

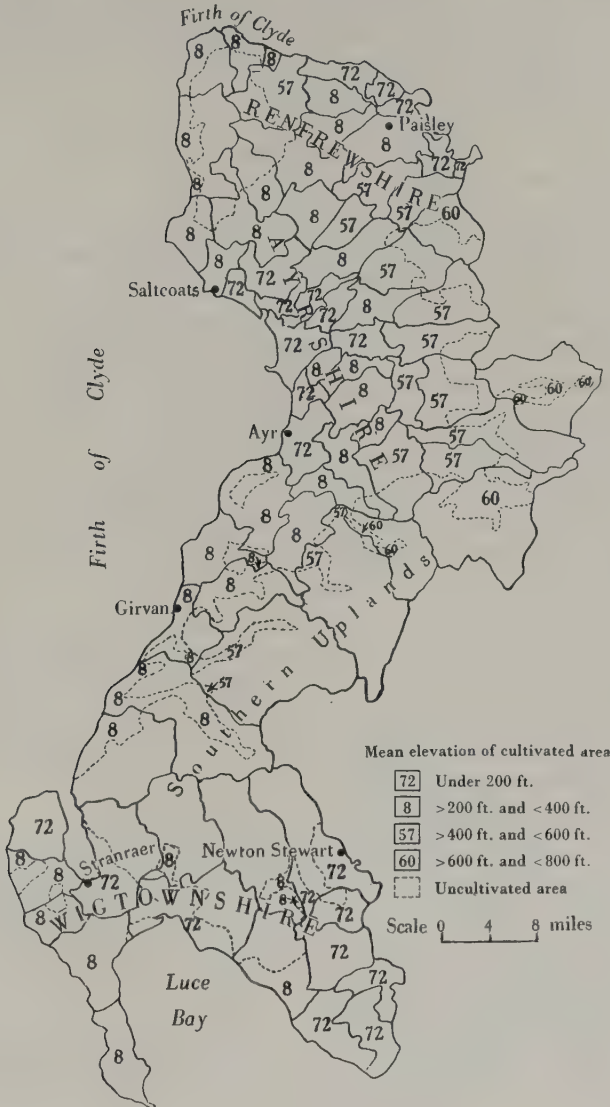


Fig. 1c. Region 3.

sula of Wigtown where much of the surface has been scraped by the ice. There are belts of alluvium of varying width in the valleys, raised beaches

round the coast, especially in 1 and 3 (sometimes covered with blown sand and only fitted for rough grazing), and numerous stretches of sand and gravel, the largest being in the lower parts of Strathmore, the Howe of Fife and the Dee Valley; but over by far the greatest part of these regions the drift consists of boulder clay. The mean elevation of the cultivated land is illustrated in Figs. 1*a*, *b* and *c*.

(ii) *Climate.*

(a) *Temperature.*

The climate of all three regions is characterised by mild winters (the winter temperatures are abnormally high for the latitude), cool summers, slow warming in spring, especially in Region 2, and slow cooling in autumn. Both the annual and diurnal ranges of temperature are small. The estimated accumulated temperatures are given in Figs. 2*a*, *b* and *c*.

The main factors influencing the temperature distribution are:

(1) *Position in relation to the prevailing winds and the seaboard.* This is specially important in winter when the isotherms run approximately from north to south. In January the coastlands of Ayr and Wigtown have average temperatures exceeding 39° F., while the sea-level temperature in many parts of regions 1 and 2, sheltered from western influences, is less than 38° F.

(2) *Latitude.* This is more important in the summer months when, south of the Moray Firth, the isotherms run in curves convex to the north, the curvature increasing from April to July. Consequently during these months the interior is warmer than the coast, and along the east coast the temperature decreases northwards, giving cold springs and late seasons in Aberdeenshire.

(3) *Altitude.* In a small region of greatly varied height this is very important. In calculating the approximate mean temperatures for parishes, the mean elevation and the standard fall of 1° F. for every 300 ft. rise were used (the temperature difference between the stations at Leith and Blackford Hill, Edinburgh, actually corresponds to the standard).

(4) *Local topographic conditions.* For example, the effect of exposure on temperature, and the low winter temperature of enclosed basins and valley sections due to the down settling of cold air in calm weather.

The frequency of ground frosts is determined by those factors which affect winter temperatures and may be illustrated by a few figures (see Table I).

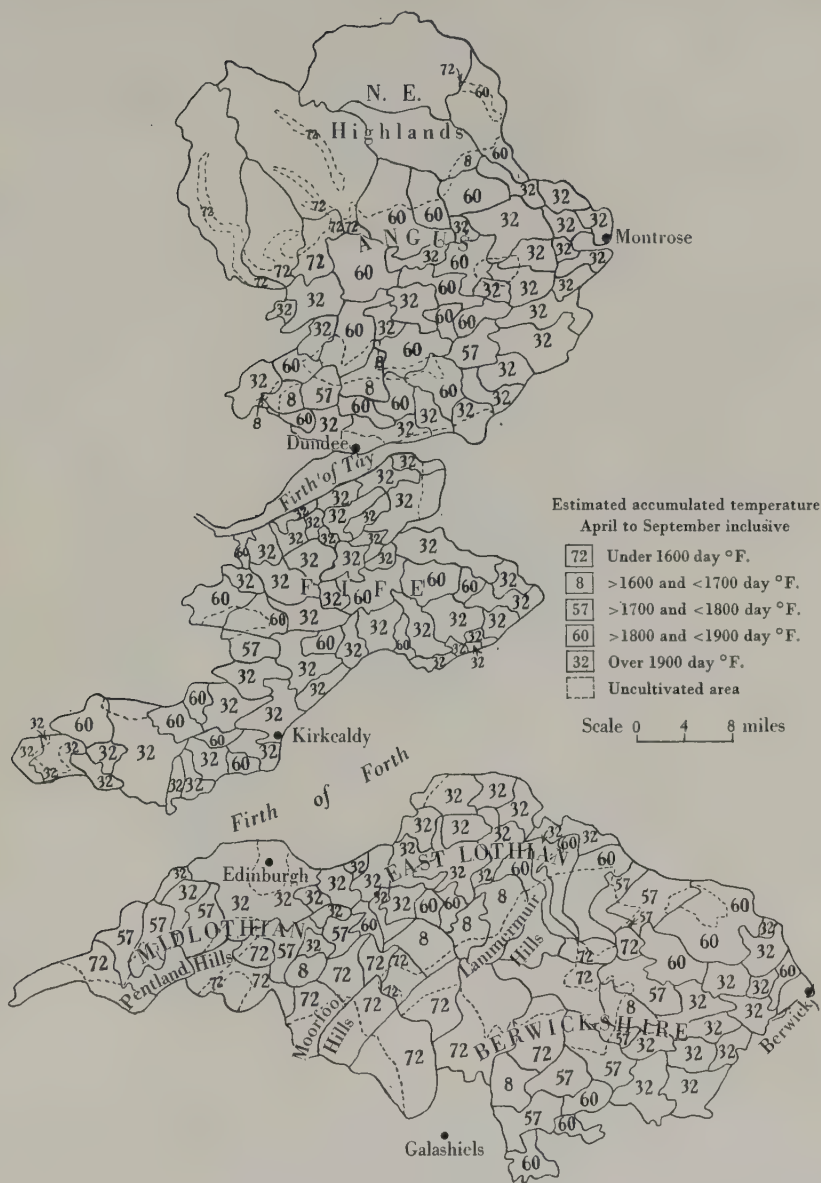
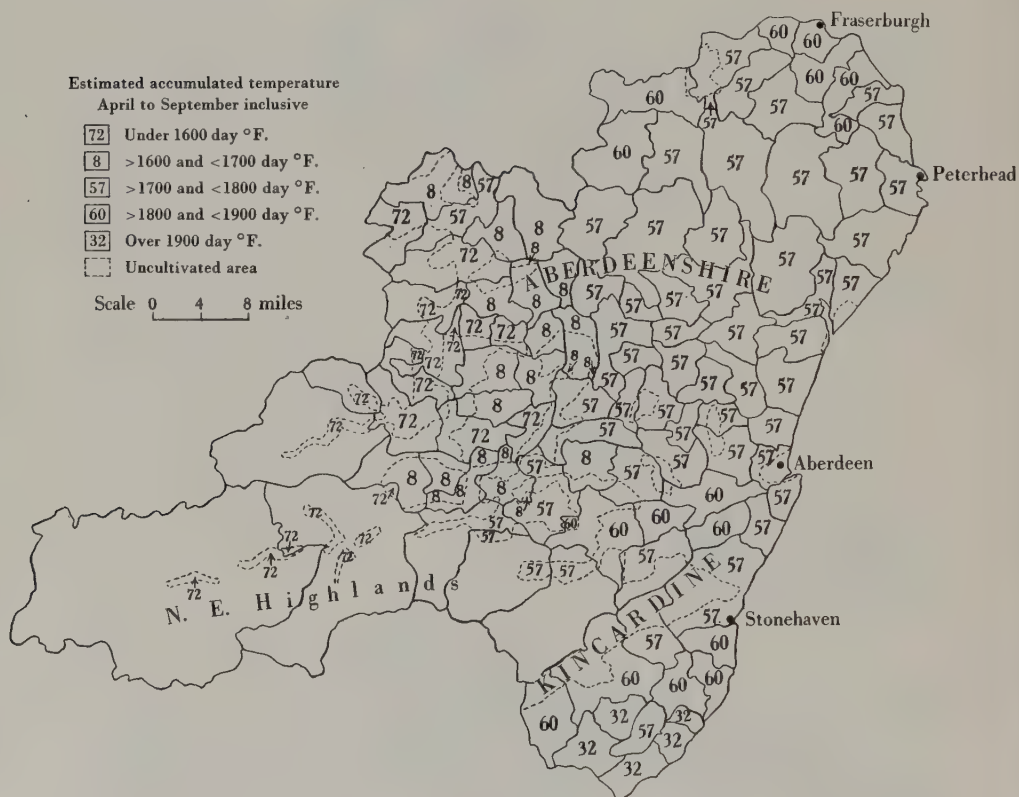


Fig. 2 a. Region 1.

Table I.

Frequency of ground frosts.

Place	Colmonell (South Ayrshire)	Edinburgh	Dundee and Arbroath	Aberdeen	Logie Coldstone (in high inland basin in west Aberdeenshire)
Number of days of ground frost	66	72	118	92	169

Fig. 2*b*. Region 2.*(b) Rainfall and sunshine.*

There is a striking positive correlation between rainfall and elevation in each region. Owing to the prevalence of westerly winds and their moisture-carrying capacity, however, the rainfalls in region 3 are much greater than those at corresponding elevations in 1 and 2. The lower parts of the Lothians and Berwickshire and a coastal strip north of the Forth have less than 20 in. per annum and the rest of the cultivated parts of 1

and 2 from 30 to 40 in. In 3 only the lowest parts have less than 40 in. and the higher may have as much as 60 in. (see Figs. 3*a*, *b* and *c*). The

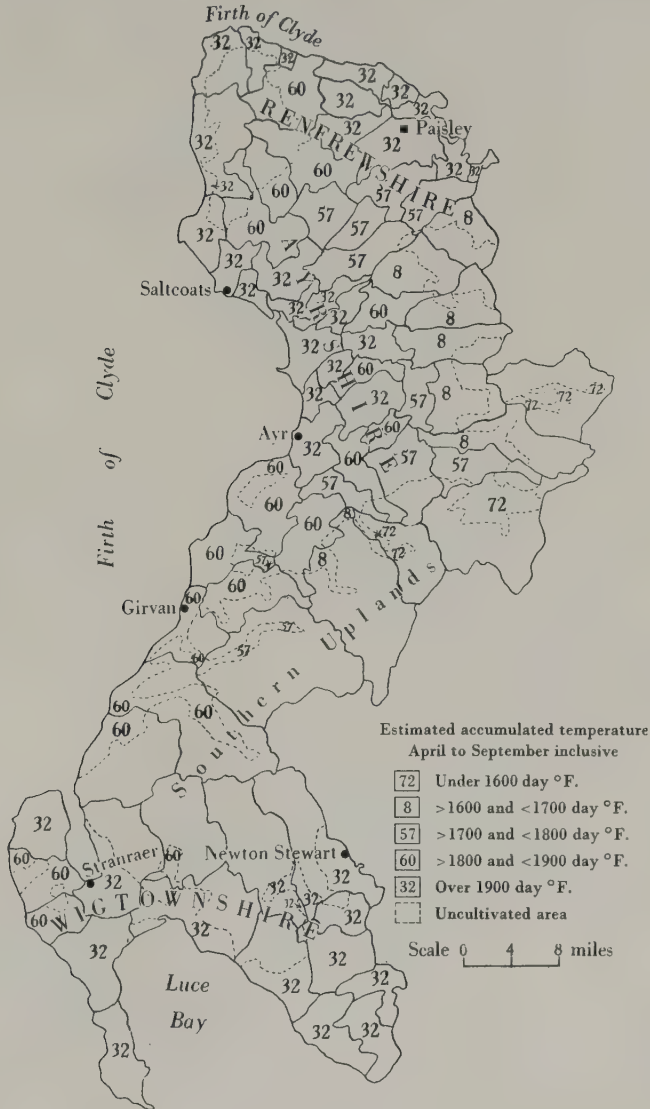


Fig. 2*c*. Region 3.

number of days with rain and the amount of cloudiness are also greatest in 3. In all regions spring is the driest season and autumn and summer

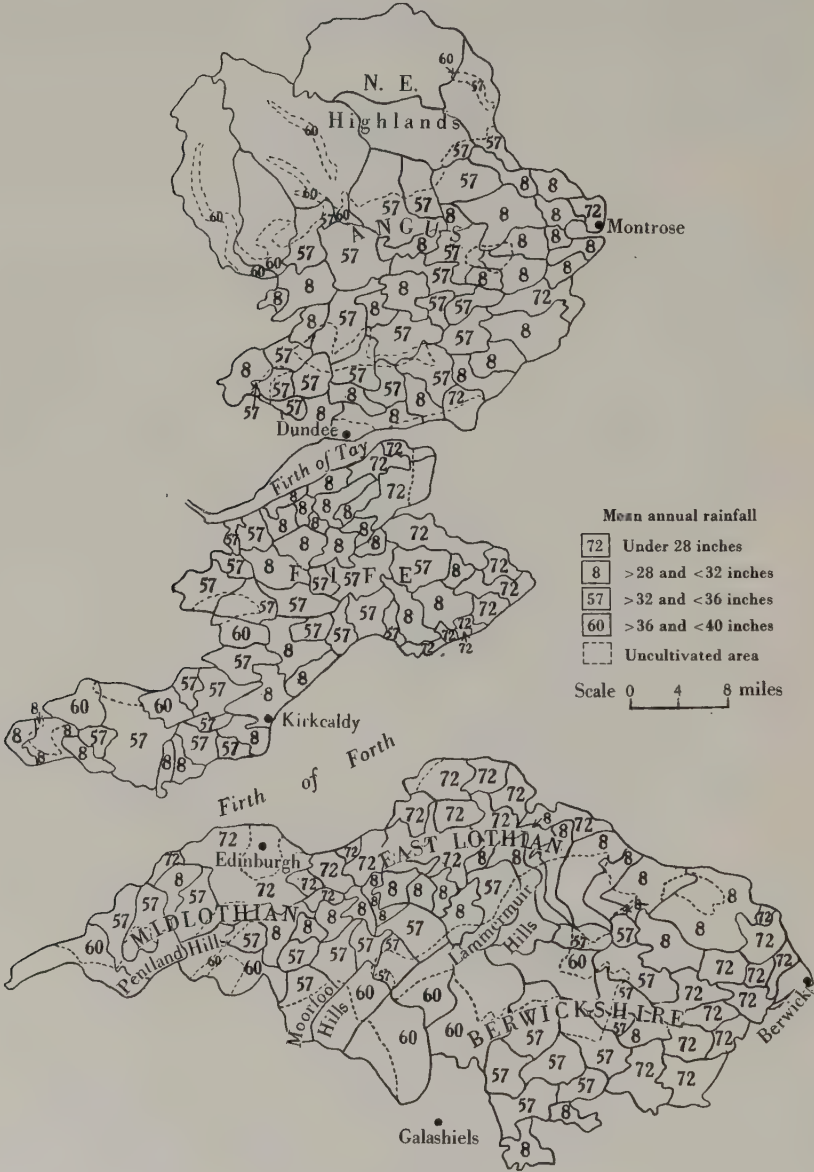


Fig. 3 a. Region 1.

are relatively wet, the July-August maximum exceeding that of autumn in the lower parts of 1 exclusive of east Berwickshire. Winter is generally the wettest season in 3 and the higher parts of 1 and 2 (see Fig. 3*d*).

According to the small number of records available the duration of sunshine in Scotland appears to decrease in a north-westerly direction. In eastern districts it is slightly greater on the coasts than in the interior.



Fig. 3*b*. Region 2.

(iii) Soils.

The absence of a soil survey or of any systematic and detailed information on soils was a serious drawback in this investigation, especially in the fairly numerous cases where the soil factor seemed of greatest importance. Thus, with few exceptions, no tabulated material could be drawn up for individual parishes. For information recourse had to be made to geological memoirs, articles by soil scientists, regional studies and general works on agricultural geology.

From the point of view of broad climatic classification the majority of Scottish soils belong to the podsol group, since the prevailing humid

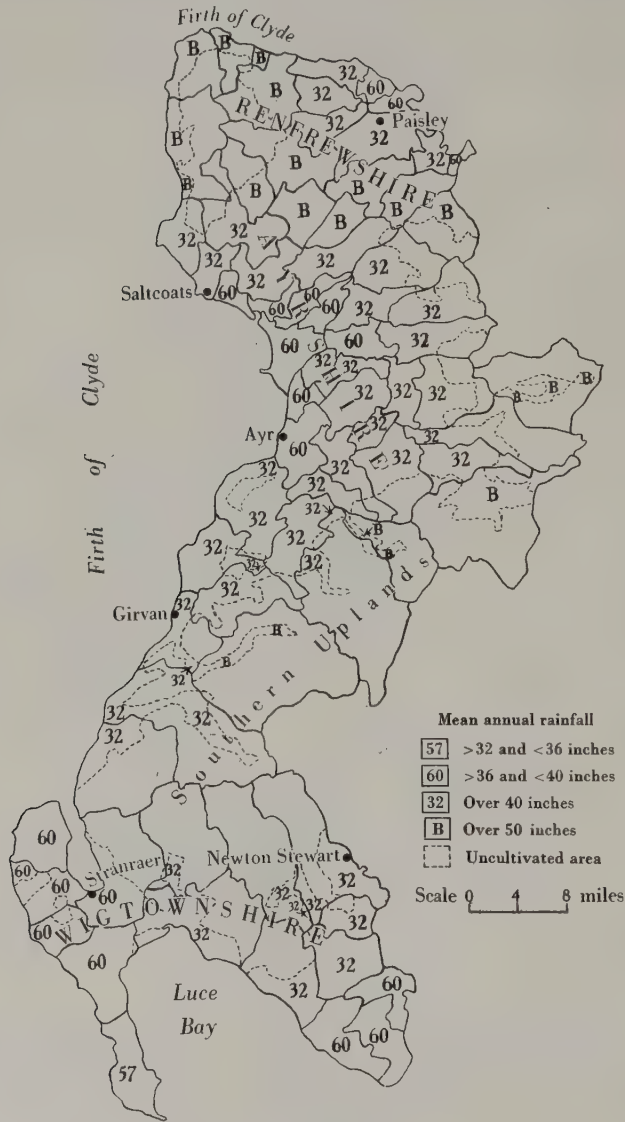


Fig. 3c. Region 3.

conditions have given rise to a leaching of bases from the surface layers and a development of acidity. In eastern Scotland, other things being

equal, acidity is greater at the higher elevations and on light soils with free drainage (Ogg and Dow(10)). The mineralogical composition of the soil varies according to the nature of the parent rock even when there is a covering of boulder clay, for the matrix of the latter is generally made up of the disintegrated materials of the rock immediately traversed by the ice. Exceptions occur in narrow contact zones when the ice moved more or less at right angles to the outcrop (Hart(3), Hendrick and Newlands(4, 5)). In eastern Scotland soils derived from boulder clay over rocks of the Carboniferous Limestone Series are the most basic, being frequently alkaline, and those over basic volcanic rock, alluvium and Old Red Sandstone conglomerate are as a rule less acid than the remainder (Ogg and Dow(10)). In Aberdeenshire many soils with acid reactions have given good crops for years. But these soils, formed on mechanically weathered glacial drift, are characterised by a relatively



Fig. 3d. Typical rainfall regimes.

high content of undecomposed ferro-silicates containing large reserves of bases suitable for plant food, and these become slowly available for crops. This is partly true of all Scottish soils except those on the Carboniferous formations which resemble more closely the soils of south-eastern England in their high content of the residual products of chemical weathering (Hendrick and Newlands(4, 5), Hendrick and Ogg(6)).

The soils developed on drift vary in texture according to its nature. Those on alluvium are varied, but usually of a sandy or silty nature, those on the sand and gravel deposits and the raised beaches, except in the limited areas of brick clay, are generally light. Though on the whole stiff and retentive, the soils on the boulder clay vary considerably, especially on the Carboniferous formations. Nevertheless, as a consequence of the general relationship between the boulder clay and the underlying rock, they show a general variation with different rock types, *e.g.* on the Old Red Sandstone they are generally red and sandy, on the

Coal Measures stiff and stony. Where there is no drift cover the soils vary more closely with the nature of the underlying rock, *e.g.* on basic igneous rock they are generally warm light free-working loams (Monie (9)).

III. LENGTH OF GROWING SEASON.

If account be taken only of air temperature, the length of growing season at different places can be roughly compared by calculating from the monthly means the accumulated temperature above 42° F. during the months when the principal crops are in the ground (see Figs. 2*a*, *b* and *c*). Naturally it varies with the same factors as those influencing spring and summer temperatures. An interesting feature in all regions is the prolongation of "growing temperatures" into the autumn, a condition suitable for the turnip crop. But even if other conditions were favourable, it would not be practicable to take advantage of these relatively high autumn temperatures for extending the areas of cereals or potatoes at high elevations owing to the prevalence of storms, the high rainfall, and the occurrence of early frosts during that season.

But air temperature is by no means the only factor involved. Variations in soil conditions through their influence on soil temperature also play a very important part, especially where the spring rise in air temperature is slow or the available amount of heat near the lower limit for any crop. Thus, other things being equal, a sandy soil will warm up more quickly in spring than one of a heavier nature, partly because its specific heat is less than that of loam or clay, but even more because water drains quickly from it, for the specific heat of water is from five to ten times that of any soil. The lateness of a heavy soil is in large part due to the utilisation of much of the available heat for evaporating the contained moisture before any rise in temperature can take place (Hall (2)). If the rainfall is high and the drainage poor the evil effect is intensified. Also, in wet districts light soils can be prepared earlier in the season and the resulting warming and aeration promotes the nitrification by soil bacteria necessary for growth. The crops can thus be sown earlier. Owing to their greater absorption of heat dark-coloured soils warm up more quickly than light-coloured, while the presence of humus, on account of its black colour and continual fermentation, also accelerates warming (Hall (2)).

IV. INFLUENCE OF PHYSICAL CONDITIONS ON KIND OF CROP AND LENGTH OF ROTATION.

The effect of the physical conditions on the various crops, classes of livestock and types of farming practice was studied by mapping, grouping, and other statistical methods. In the case of the principal crops a

Table II.

Distribution and physical conditions of crop-growing areas.

Crop	Areas of greatest acreage	Areas where absent or little grown	Favourable environmental factors		Unfavourable environmental factors		Physical conditions in area of maximum acreage
			Long growing season	Possibility of following potatoes	A.T. (Apr.-Aug.) less than 1480 d.d.F., there-fore high elevation	A.T. (Apr.-Aug.) 1650-1750 d.d.F.	
Wheat	Lower parts of Midlothian and Angus North-east Fife and coastal districts of East Neuk Lowest part of Paisley lowland	Aberdeen and North Kincardine (a) Wigtown and South Ayr (a). Rest of Ayr and higher parts of Renfrew Berwick and higher parts of Lothians, Fife, North Angus and South Kincardine			High rainfall in autumn	Rainfall 25-27 in.	Soils—low acidity, variable texture
Barley	Lower East Lothian, especially near the coast East Berwick, especially in lower parts Coastal fringe of East Fife and East Angus Lower parts of Lothians, Angus and East Fife, especially near the coast and near Edinburgh and Dundee Coastal fringe of Ayr and lowest part of Paisley lowland	Region 3 (a) Region 2, except lower parts in south-east Midlothian and western parts of Berwick, Fife and Angus Higher Lothians, Berwick, West Fife, Highland parishes of Angus Region 2, except near Aberdeen and in extreme south-east Region 3, except parts in preceding column	Low rainfall A.T. (Apr.-Aug.) greater than 1550 d.d.F. Light soils Proximity to sea		Heavy soil when temperature conditions are limiting or rainfall high High rainfall (v) Acidity of soil (v) Too rich soil due to heavy manuring in neighbourhood of cities	A.T. (Apr.-Aug.) 1650-1750 d.d.F. Rainfall 24-26 in. Soils—light with low degree of acidity	
Potatoes			Light soils (best quality on Old Red Sandstone loams) Proximity to population centres. Good communications Forearlies—in addition—freedom from spring frosts		Heavy soils (v) Cold soils High rainfall, except where soil is very porous	A.T. (May-Oct.) 1900-2000 d.d.F. Rainfall 25-27 in. (on porous soils of Ayr coast 37-40 in.) Soils—variable but mainly light	
Oats	Aberdeen and Kincardine Medium elevations in Angus West Wigtown	Highest and wettest parts of region 3	General cool, moist conditions, suitable almost throughout. Large percentage of arable land with little competition from wheat, barley or potatoes Light soils Cool summers		Very high rainfall, both directly and through its effect on the arable area	Optimum conditions A.T. (Apr.-Aug.) 1450-1750 d.d.F. Rainfall 27-35 in. (Ref. Geddes (1))	
Turnips	Aberdeen and Kincardine Most of Angus Some of lower parts of East Lothian and Berwick	Ayr, Renfrew and East Wigtown Highest parts of region 1 South-west Fife and West Midlothian	Absence of competition from potatoes and other vegetables Very low rainfall		Heavy soils (v) Acid soils Rainfall exceeding 37 in. (or less if soil is on heavy side) Very low rainfall	Optimum conditions A.T. (June-Nov.) 1450-1750 d.d.F. July temp. 54-57° F. Rainfall 28-35 in. Soils—light (Ref. Geddes (1))	

Abbreviations: (a) absent or nearly so; (v) very unfavourable; A.T. accumulated temperature; d.d.F. day degrees Fahrenheit.

discussion of the results and the processes by which they were obtained has already been published with maps (Snodgrass (11)). These results are summarised in Table II.

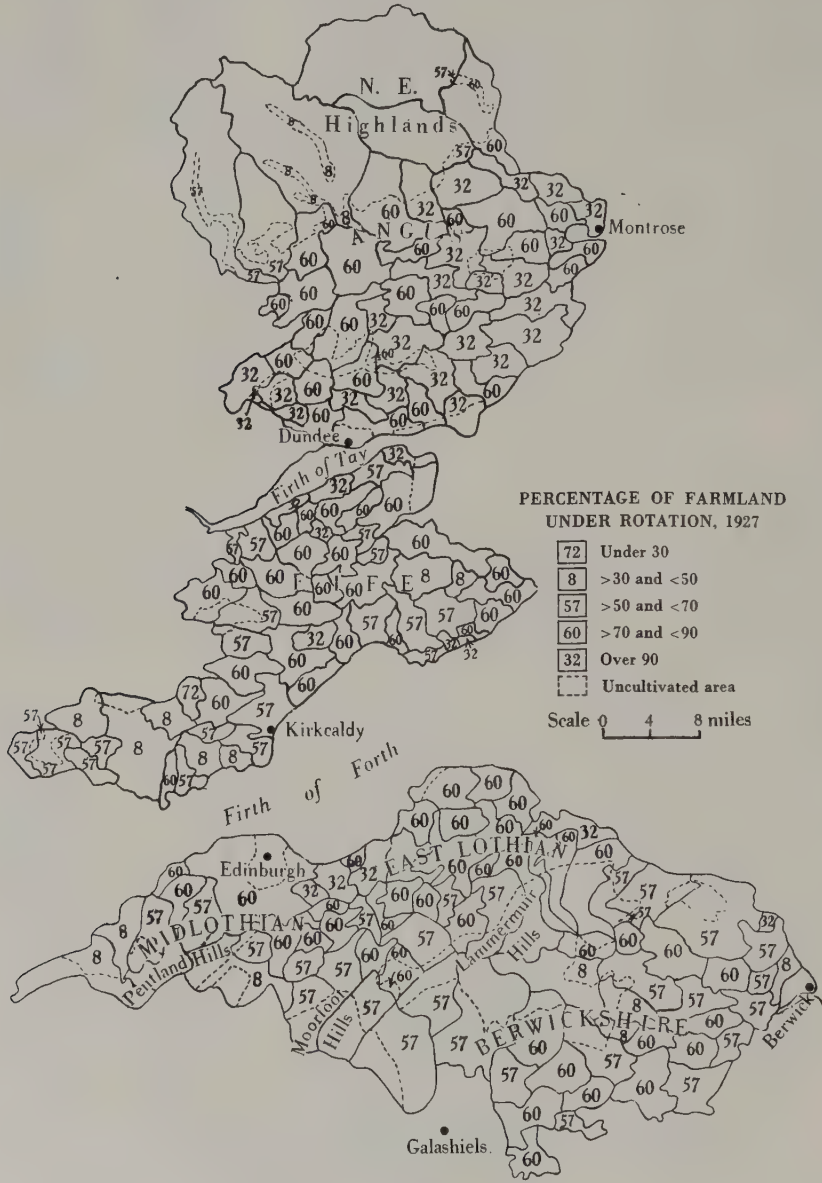


Fig. 4a. Region 1.

The percentage of farm land under rotation is illustrated in Figs. 4*a*, *b* and *c*, while Figs. 5*a*, *b* and *c* show the length of rotation expressed as a percentage of the arable area occupied by crops other than rotation grass. It was found that rotations of over 75 per cent. occur only in region 1, where the most extensive area of close cropping is found in the belt of lowland stretching from Kirkliston through Edinburgh to Dunbar,

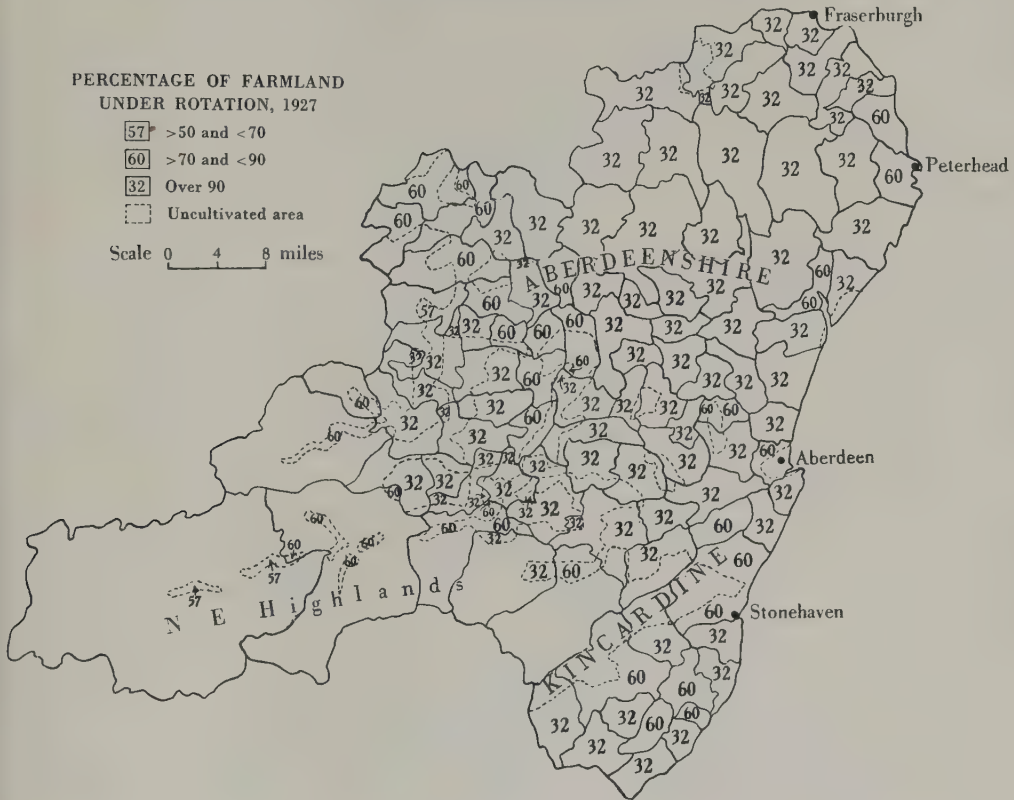


Fig. 4*b*. Region 2.

and the next on the south coast of the East Neuk of Fife. Those of under 40 per cent. are practised in the higher and some of the lower parishes of Ayr and Renfrew, in south-east Wigtown and the highest parishes of East Lothian and Berwick. Intermediate rotations whose length varies with changing physical and economic conditions prevail elsewhere. In Aberdeenshire the uniformity is remarkable, approximately half the arable area being in rotation grass throughout.

The contrast between regions 1 and 3 at corresponding levels and the limitation of short rotations to the driest parts of 1 bring out clearly the

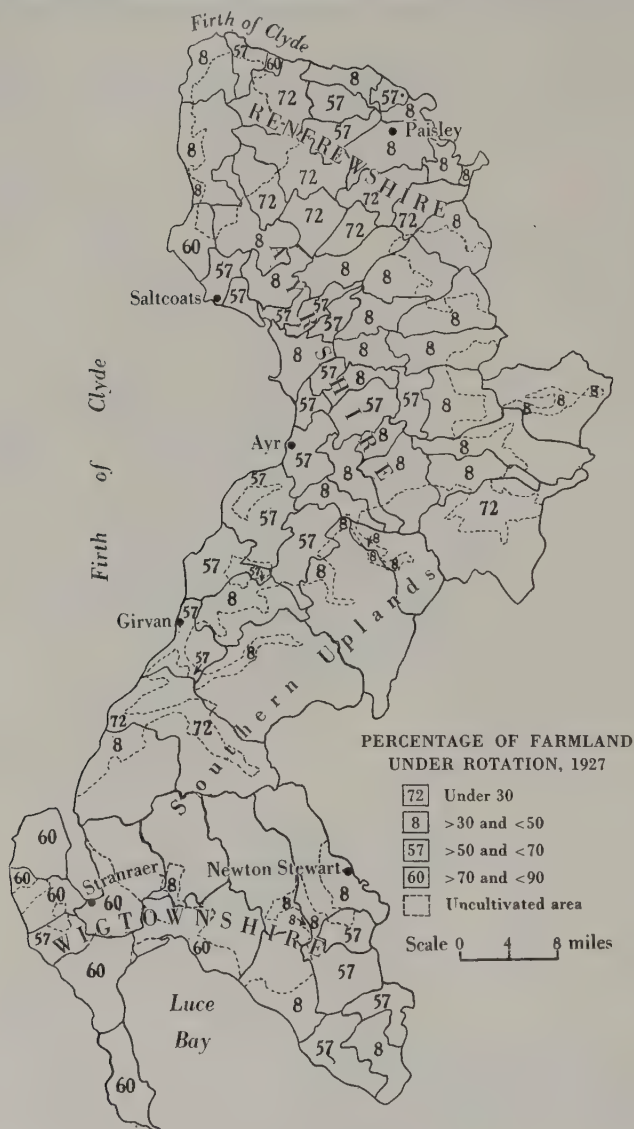


Fig. 4c. Region 3.

effect of high rainfall. It is favourable to grass but detrimental to grain and roots, especially at some seasons. In 1 and 3 shorter rotations are

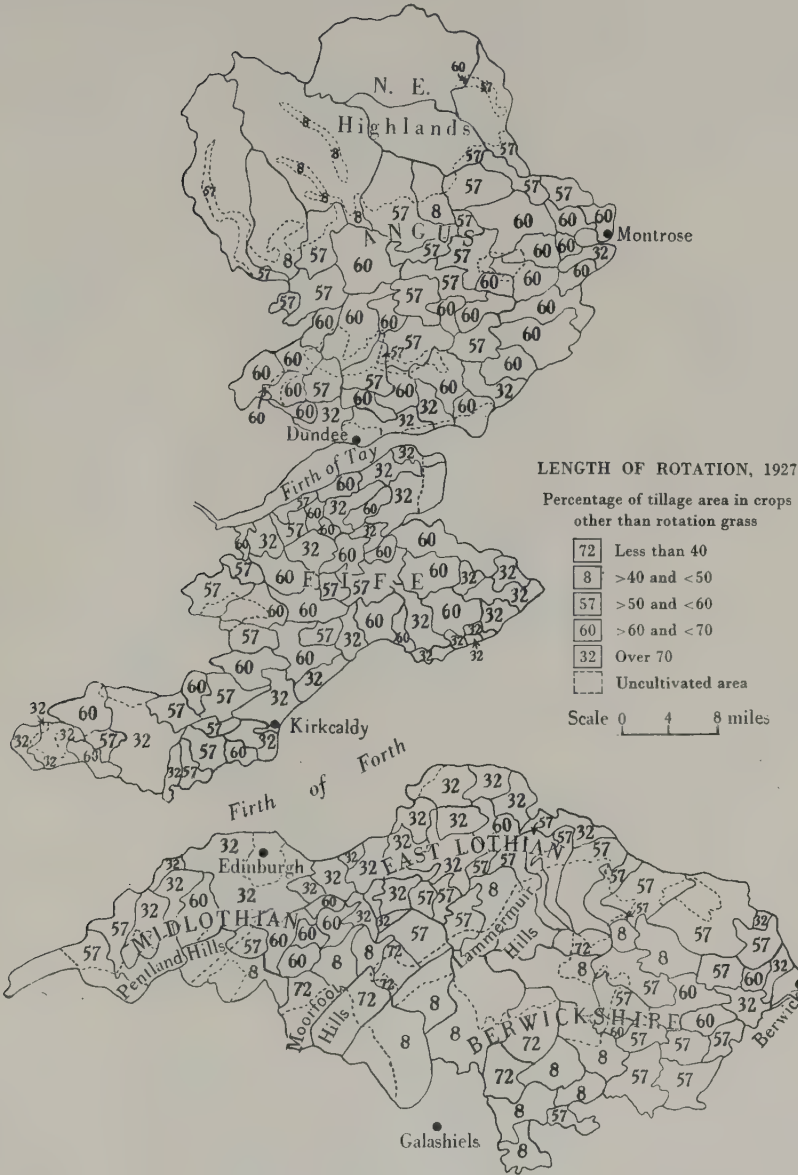


Fig. 5 a. Region 1.

practised on the whole in the lower and longer in the higher parishes, East Lothian showing a distinct zoning according to elevation. This is due among other things to the short growing season, stormy autumns and general unsuitability of the higher parts for such crops as wheat, barley and potatoes which are prominent in the short rotations at the lower levels; also to the acidity of soil which precludes the too frequent growing

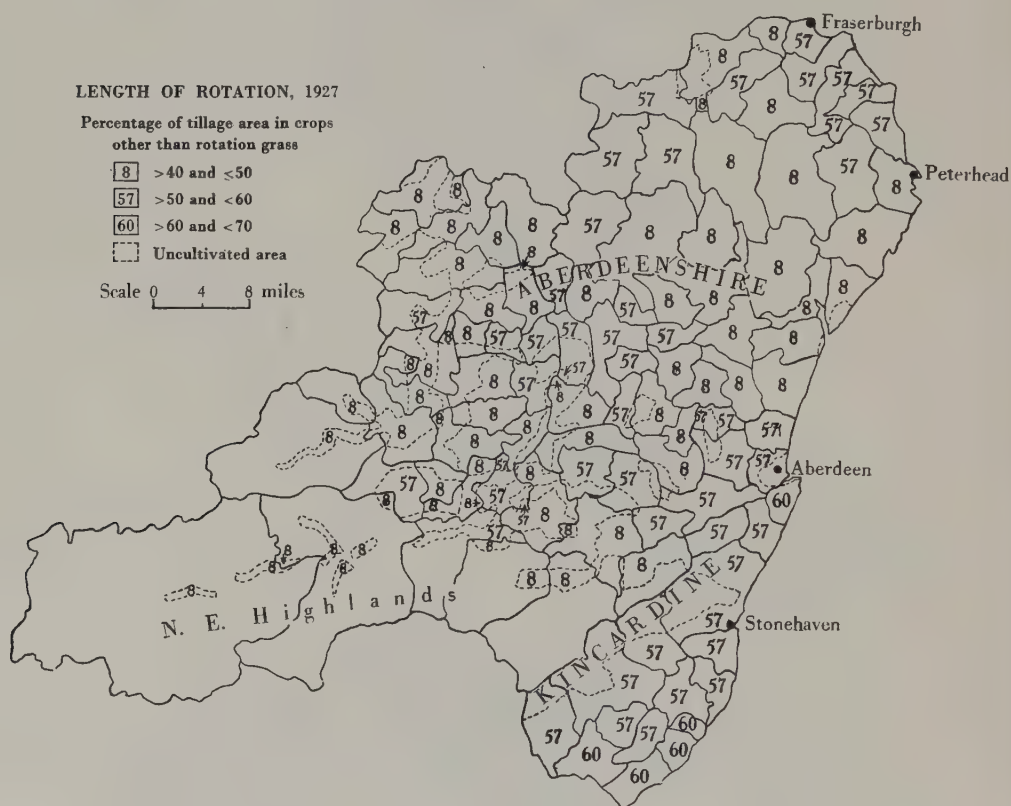


Fig. 5b. Region 2.

of turnips. In Aberdeenshire and north Kincardineshire, on the other hand, there is no such effect. There, sale crops other than oats play a very minor part, even at low elevations, while the uniformity of farming system results in a fairly equal demand throughout for winter fodder (oats and turnips) for the livestock. The effect of proximity to large centres of population in shortening the rotation, *i.e.* intensifying the agriculture if the amount of permanent grass does not increase, is shown

by the distribution of relatively short rotations in all these regions, and that of nearness to the sea and inherent fertility of the soil in 1 and 3.

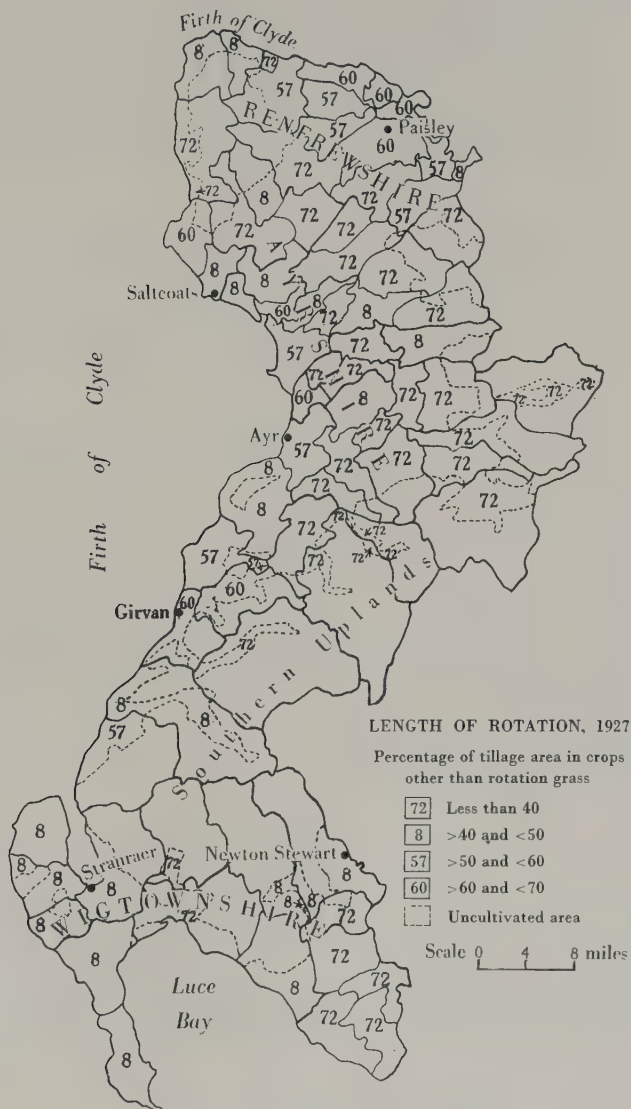


Fig. 5c. Region 3.

V. SUMMARY.

1. The co-ordination of the available data relating to the environmental conditions of agricultural regions is advocated as a means of facilitating the testing and ultimate distribution of new crop varieties.

2. The physical environment (topographical, geological, climatic and edaphic) of three agricultural regions is described, and its influence on the length of the growing season, the length of crop rotation, and the distribution of the principal crops is discussed.

3. Taking the parish as the unit, maps for the three regions are given to illustrate the mean elevation of cultivated land (Figs. 1*a*, *b* and *c*), the estimated accumulated temperatures (Figs. 2*a*, *b* and *c*), the mean annual rainfall (Figs. 3*a*, *b*, *c* and *d*), the percentage of farm land under rotation (Figs. 4*a*, *b* and *c*) and the length of rotation (Figs. 5*a*, *b*, and *c*).

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THE OCCURRENCE OF COPPER POISONING IN A GLASSHOUSE CROP

By JOHN CALDWELL.

(Rothamsted Experimental Station, Harpenden, Herts.)

(With Plate XX.)

THE toxic effect on plants of salts of the heavy metals has been studied at some length and much information on the subject is available. The purpose of this paper is to direct attention to an instance in which the effect of the toxicity of copper was to induce in cucumbers and in tomatoes a condition which on first inspection very closely resembled a virus disease. About the end of January 1935 I was asked to examine some cucumber seedlings at a commercial nursery. The information given was that out of 1500 cucumber seedlings which had been "potted up" in "thumb 60" pots, 1000 or so had been discarded as it was thought they showed symptoms of mosaic. It was reported that similar symptoms had manifested themselves in the previous year and the affected plants had been burnt, and that it was only at this nursery and not at the firm's other branches that the trouble had been observed. An examination of the affected plants was made and experiments set up to discover the cause of the complaint. The relevant data obtained are given below.

The general appearance of the affected seedlings suggested that they were suffering from a virus disease. The leaves were distorted and chlorotic and there was marked "clearing of the veins". In the most affected plants some necrosis of the laminae was noted. The evidence against the trouble being caused by a virus was the improbability of there being any source of infection at that early date, coupled with the fact that the same seed was used in all the different nurseries of the firm. It was found that removal of the seedlings from the "thumb 60" pots into "48's", filled with fresh soil, resulted in the complete recovery of the plants, the new leaves being quite normal, though the previously affected leaves remained affected. This observation, coupled with those above noted and supported by the fact that the disease was not, apparently, transmissible to healthy cucumbers in our glasshouses, indicated that a virus was probably not the cause of the trouble. It was thought that the soil or the conditions of cultivation might be responsible and experiments

were set up, both at the nursery and in the Rothamsted glasshouses, to examine this point. Five sets of plants, grown in boxes and subsequently pricked out into "thumb 60" pots, were set up in the nursery. Each group was planted in soil slightly differently treated. The seed boxes in which the seedlings were raised were filled with sterilised used soil. The seedlings were transplanted from the boxes into pots which were divided into five groups as follows: (a) 2 parts sterilised old soil and 1 part of sterilised "maiden" soil, (b) 2 parts of sterilised old soil and 1 part of unsterilised "maiden" soil, (c) 2 parts of sterilised old soil and 1 part of sterilised maiden soil (the pots to be used having been reboiled), (d) all sterilised old soil, (e) 2 parts sterilised old soil and 1 part sterilised "maiden" soil—put into pots which had not been boiled. In another experiment the seed was put directly into "thumb 60" pots filled with sterilised soil so that the seedlings were not touched by hand, and the pots were specially reboiled. In the Rothamsted glasshouses similar experiments were set up, using unboiled pots and pots which had been brought from the nursery after boiling. Two seeds were put into each of the pots and one group was grown in a very warm house. Half of the pots in this house stood in saucers full of water so that the soil was kept waterlogged while the soil in the other half was watered at intervals of two or three days so that it was as dry as possible. Finally, 200 boiled "thumb 60" pots from the nursery were taken to another nursery where the trouble had not previously been observed and were used under their conditions by the staff of the second nursery.

When the first foliage leaf had developed the plants were examined, and it was found that about 25 per cent. of the plants grown in the nursery under treatments (a), (b), (c) and (d) were affected with the chlorosis while the plants under (e), grown in unboiled pots, were normal. The seedlings grown in the "thumb 60" pots without pricking out were 90 per cent. affected by the chlorosis. Under Rothamsted conditions, neither seeds from the nursery nor from our own source showed any sign of chlorosis when grown in our pots either under wet or dry or warm or cool conditions, but a variable number of the seedlings grown in the pots from the nursery showed the typical symptoms. While it was noted that the plants in the waterlogged soils tended to be softer than the others and were definitely chlorotic, the chlorosis was generalised and quite unlike that symptomatic of the condition under examination. It was noticed, also, that in our glasshouses, either both of the seedlings in the pot were normal or both were affected—in no event was one affected and the other normal. More than fifty of the plants grown in the

other nursery showed the typical symptoms which were under observation.

It was concluded from these observations that neither the dry nor the wet conditions could of themselves induce this chlorosis, nor were the soil and the conditions of growth responsible. The boiling of the pots appeared to be the source of the trouble, and since it was assumed that boiling *per se* was unlikely to render the pots toxic to plants, the question of the type of boiler used was raised. It was found that three small boilers were in use, two of cast iron and one of copper. The copper boiler had been "tinned", but about two and a half years ago it had been repaired and it was evident that the repair had been made with "untinned" sheet copper. It was suggested that copper poisoning was the source of the trouble, which had by now been discovered among the tomatoes of the same nursery, and it was recommended that some alteration in the system of sterilising pots be introduced. Apparently, the close association of the roots of the plants with the sides of the pots made available the small traces of copper contained in them.

At the same time, to demonstrate that copper was in fact the cause of the trouble, a series of experiments was set up in the Rothamsted glasshouses. A group of cucumber plants was treated with 1/100 copper nitrate solution which was absorbed through a cut petiolar stump inserted into a small tube containing about 2 c.c. of solution. Those plants which absorbed more than 1 c.c. of the solution usually died after a few days, while some of the leaves of those which absorbed only 0.5 c.c. were distorted and yellow, the effect of the copper being not unlike that observed in the original plants. Another group of plants in pots were watered with 1 per cent. copper sulphate solution. This was given in doses of 25, 50 and 100 c.c. respectively on five successive days, but no very marked symptoms developed. In another group of pots there was added to the soil as a layer in the lower part of the pot copper carbonate, and into each pot were put seeds. When the seedling developed the first foliage leaves it was found that, in many instances, chlorotic symptoms developed not unlike those reported above.

An attempt was made to grow seedlings in water cultures containing copper in solution. Seedling cucumbers were put into Knop solution containing (a) no added copper, (b) 1/1000 copper nitrate, (c) 1/2000 copper nitrate and (d) 1/5000 copper nitrate. After three days the seedlings of treatments (b), (c) and (d) were transferred to ordinary Knop solution. The stems of all the treated seedlings were somewhat shrivelled, especially in the region of the hypocotyl, and little growth had been

made. In the Knop solution some recovery of the seedlings of treatments (c) and (d) took place and the foliage leaves of those of (d) grew sufficiently for it to be obvious that they were distorted and chlorotic, especially round the veins. It was clear, therefore, that the effect of the addition of copper to the solution had been to induce symptoms similar to those observed in the original cucumber plants.

CONCLUSION.

From these experiments it is concluded that small traces of copper have the effect of inducing in cucumber plants, under appropriate conditions, a disease which symptomatically is not unlike a virus disease. There is evidence that the disease is not, in fact, transmissible, nor is it in any way connected with the presence of a virus. Finally, the observations made in this investigation in which cucumber and tomato plants have been involved indicate that great care should be exercised to preclude the possibility of toxic quantities of copper being present in pots and soils in commercial houses and illustrate the errors which may arise in observations on diseased material when traces of toxic substances are present.

EXPLANATION OF PLATE XX.

- Fig. 1. Cucumber plants grown under wet conditions. (a) healthy, (b) affected.
Fig. 2. Cucumber plants grown under dry conditions. (a) healthy, (b) affected.
Fig. 3. Two cucumber plants grown as seedlings in treated pots. (a) left in same pot, (b) transferred to clean pot.

(Received March 5th, 1935.)



Fig. 1.



Fig. 2.

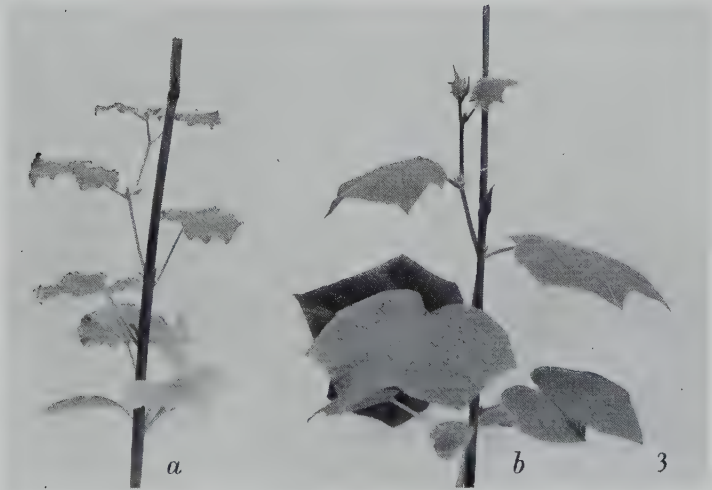


Fig. 3.

POTATO BLIGHT (*PHYTOPHTHORA INFESTANS*) INVESTIGATIONS IN JERSEY. PREVENTION OF DISEASE IN EXPORT PRODUCE

BY T. SMALL, PH.D., A.R.C.S.

(*States Experimental Station, Jersey, C.I.*)

THE prevention of blight (*Phytophthora infestans* (Mont.) de Bary.) in seed potatoes has been dealt with in a previous publication (2). The aim of the present investigation was to eliminate the disease from produce grown for export to England. This produce consists of early potatoes which must be dug and marketed as soon as possible to obtain remunerative prices. At harvesting time the haulms are still green and growing, and the tubers are immature, have a thin skin and a low specific gravity. It will be seen, therefore, that the potato-blight problem in Jersey differs from that connected with late potato crops in which the mature tubers are usually dug after the haulms have died down and the produce stored before it is marketed.

Only one variety, International Kidney, is grown and this is very susceptible. Sometimes, as in 1921 and 1934, the crop remains healthy, but occasionally, as in 1926, it is rapidly destroyed. More usually, however, the disease appears in May but is not serious until mid-June, and crops harvested towards the end of the season, *i.e.* late June and early July, are liable to suffer most from blight.

Decreased yields due to foliage attack are not usually important. The chief loss arises from the fact that tubers which are apparently sound when packed for export often develop disease in transit. The appearance of such produce in England leads to decreased demand and lower prices; the loss is not confined to the particular grower but is shared by growers who are marketing sound produce. Another but less important source of loss is the diseased tubers present at digging time. Under careful supervision these potatoes are rejected in the field and do not, therefore, affect prices and markets.

LOSS IN TRANSIT.

(1) *Development of blight in tubers subsequent to harvesting.*

Apparently healthy tubers, dug from crops in various stages of disease, were stored in barrels and examined two weeks later. Typical results obtained in 1932 and 1933 are given in Table I.

Table I.

Development of blight in tubers subsequent to digging.

Condition of haulms at digging time	Tubers		
	No. healthy	No. diseased	Diseased %
Disease severe on partly green and partly dead haulms	47	147	76
" "	38	66	63
" "	55	65	54
" "	42	48	53
" "	270	173	39
Little disease on haulms	378	43	10
" "	423	44	9
Haulms killed by " disease; brown and dry	92	12	12
" "	52	0	0
" "	113	0	0
Haulms healthy	93	0	0

Tubers from healthy crops remained sound; where the haulms had been killed by disease the tubers usually remained healthy but occasionally a few developed disease; a little blight on the haulms resulted in some disease in the potatoes, but the greatest loss occurred where the disease was prevalent on the partly green and partly dead foliage.

These results suggested that the disease which develops in transit is due to (a) contamination of healthy tubers by spores falling from diseased haulms at digging time as shown by Murphy and McKay (1), and (b) packing tubers which are infected, but not obviously, when dug. Approximate relative values for these two sources may be ascertained by immersing a sample of freshly dug tubers in formaldehyde as described elsewhere (2). This kills the spores which fall on the tubers, so that any disease which develops indicates that the tubers were infected when dug. This treatment practically prevented disease in tubers harvested when the blight attack was prevalent but recent; untreated tubers developed much disease as shown in Table I. On the other hand, the disease was not appreciably reduced by dipping tubers dug after the haulms were dead. It is clear, therefore, that the development of blight in tubers subsequent to digging is due mainly to spores falling from diseased haulms in the case of recent attacks, and to incipient infections present in the tubers at digging time where the attack on the haulms has reached an advanced stage before the crop is harvested. The former is by far the more serious source of loss. It may be noted here that mere shaking of diseased foliage over healthy tubers is sufficient to cause disease in the latter; contact is not essential.

(2) *Spread of disease in transit.*

Experiments were made to determine if the disease spread to healthy tubers when these were packed with diseased or contaminated tubers. Marked, diseased potatoes were packed among sound tubers in one barrel, while in two other barrels marked healthy tubers which had been inoculated with a spore suspension and allowed to dry for an hour were packed among healthy tubers. A control barrel contained only healthy potatoes dug at the same time and from the same plot as the other healthy tubers used in the experiment. The barrels were shipped to Weymouth and returned to Jersey for examination. The results are given in Table II and show that the disease spread from diseased or contaminated tubers during transit.

Table II.
Spread of disease in barrels.

Barrel	No. of inoculated or diseased tubers placed in barrels (27. vi. 32)	No. of diseased tubers (11. vii. 32)	No. of inoculated tubers still healthy	No. of tubers to which disease spread in transit
1	0 (controls)	2	—	2
2	70 inoculated	78	23	31
3	70 „	96	13	39
4	63 diseased	106	—	43

(3) *Contaminated barrels.*

Returnable potato barrels are often used twice in a season which lasts only five to six weeks. It was necessary to ascertain if the fungus persisted in barrels which had previously contained diseased produce. Two barrels were filled with blighted tubers and left for seven days; they were then emptied and after four days were filled with newly dug sound potatoes. Two weeks later no disease was present in the barrels. The experiment was repeated and similar results were obtained. It is concluded that there is little, if any, danger in using barrels which have contained diseased produce.

LOSS IN THE FIELD.

This includes diseased tubers left in the field and is important only where the disease on the haulms has reached an advanced stage before the crop is harvested. It ranges from 5 to 20 per cent. of the yield and is affected by many factors, including rainfall and spraying, as shown by the following experiment.

A two-acre field was divided into strips, three of which were unsprayed and two sprayed, using a horse-drawn machine and the mixture

noted on p. 472. Blight appeared earlier in the unsprayed areas and rapidly killed the haulms; it appeared later in the sprayed areas and spread more slowly. It is important to note that at digging time the haulms were dead on all the plots. Although the sprayed areas gave a higher yield of sound tubers they also produced far more diseased tubers. A study of the weather conditions showed that during the period when the disease was developing on the unsprayed areas rain fell only twice, the amounts being 0.02 and 0.04 in. respectively, scarcely enough to wet the soil. When blight was developing on the sprayed plots the total rainfall was 1.24 in., which included two falls of 0.38 and 0.66 in. It is reasonable to assume that the rainfall influenced the above result. Another significant point is that the critical period when the disease is developing on the haulms is shorter in unsprayed than in sprayed crops. The longer period offers greater opportunities for rain to wash the spores into contact with the tubers.

Growers have had similar experiences to the one noted, and, judging only from the loss in the field, have concluded that spraying is detrimental. It will be shown later that such experiences are exceptional and can often be traced to imperfect or too infrequent spraying, or neglecting to take precautions when disease attacks a sprayed crop late in the season.

EFFECT OF SPRAYING.

It has been shown that the loss in the field and a portion of that in transit occurs only when the disease has reached an advanced stage on the haulms before the crop is dug. It seemed probable that these losses would be checked by spraying, but, in view of the conflicting opinions held by experienced growers, it was necessary to conduct trials in many parts of the island. The results conform with those obtained in other countries and will not therefore be given in detail. The spray was neutral to litmus and consisted of 4 lb. copper sulphate and about $1\frac{1}{8}$ lb. caustic soda in 40 gall. of water; it was applied at fortnightly intervals from early May, when the plants were 6-8 in. high, to late June, using a knapsack sprayer or a horse-drawn machine. Typical results are given in Table III and show that the treatment delayed the spread of blight. It was risky to reduce the number of applications below five, unless the weather was fine and sunny. The loss in the field was negligible on sprayed plots and was from 7 to 20 per cent. on unsprayed plots except in 1934 when no blight appeared.

It is concluded that in most seasons spraying, properly carried out, will keep the disease in check until a satisfactory yield of tubers has

formed. Once this stage has been reached the crop should be dug or the haulms should be removed so as to prevent any possibility of the disease reaching an advanced stage before the crop is harvested. In this way the losses discussed in this section would be eliminated.

Table III.
Effect of spraying on the disease.

Parish	No. of sprayings up to 28. vi. 32	Date crop inspected	Condition of crop	
			Sprayed	Unsprayed
St Ouen	5	27. v. 32	No disease	No disease
		28. vi. 32	Little disease	Killed by disease
St Mary	3	3. vi. 32	"	Little disease
		28. vi. 32	"	Almost killed by disease
St Lawrence	3	28. v. 32	No disease	No disease
		28. vi. 32	Some disease but far less than unsprayed	Severe disease
St Martin	3	28. v. 32	No disease	No disease
		24. vi. 32	Some disease but far less than unsprayed	Severe disease
Trinity	5	27. v. 32	No disease	No disease
		25. vi. 32	Little disease	Fair amount of disease

PREVENTION OF DISEASE CAUSED BY CONTAMINATION
DURING HARVESTING.

As already mentioned, the more serious aspect of the problem is the disease which occurs in transit due to the tubers becoming contaminated with spores at digging time. Spraying cannot always be relied upon to prevent this, because sprayed crops are sometimes attacked late in the season when the crops are being harvested. Four methods have been tried to reduce the risk of disease in the tubers:

A. Before harvesting:

- (1) Scorching the haulms.
- (2) Removing the haulms and spraying the ground.

B. After harvesting:

- (3) Ventilation of the packages.
- (4) Fumigation of the packages.

The first two methods and the results obtained were noted in a previous paper (2). The object is to kill the spores so that the grower may dig his potatoes almost at once without them becoming contaminated. The grower cannot afford to wait until the natural death of the haulms and spores occurs; this is practicable only in the case of late potato crops. Cutting the haulms is feasible in Jersey because the individual holding is small; trials showed that one man cut one acre in nine hours. Promising

results have been obtained by both these methods, but the best results are likely to be obtained where the diseased haulms are cut or scorched in dry weather, the ground sprayed, and the crop dug three days later. It is not advisable to cut the haulms in wet weather.

VENTILATION OF PACKAGES.

Experiments were made to determine if ventilating the packages would reduce the disease in contaminated tubers. Four types of packages, namely, barrels, paper-lined sacks, unlined sacks and wicker hampers, were used. They were packed with freshly dug, inoculated tubers, shipped to Weymouth and returned to Jersey for examination. The potatoes were inoculated in batches of 25 lb. each; this quantity was spread on a table and atomised with 15 c.c. of a stock spore suspension made from fresh diseased leaves. The batches were placed in the packages in rotation. Uninoculated tubers (controls) dug from the same plot at the same time as the above tubers remained healthy. The results of two experiments are given in Table IV and show that least disease developed in the hamper and most in the barrel. The produce in the latter was warm and moist while that in the hamper was drier and in excellent condition. It is important to note the very high percentage of disease seven days after inoculation. In a further similar trial more than 300 diseased tubers were present in each of four barrels five days after inoculation.

Table IV.
Relation of the package to disease.

Package	Exp. 1.* Tubers (1. vii. 32)			Exp. 2.† Tubers (13. vii. 32)		
	No. healthy	No. diseased	Diseased %	No. healthy	No. diseased	Diseased %
Barrel	122	496	80	125	459	78
"	124	461	79	207	420	69
Paper-lined sack	114	560	83	270	359	57
"	140	466	77	222	363	62
Unlined sack	178	467	72	332	231	41
"	298	402	57	336	296	47
Hamper	372	293	44	388	208	35
"	346	285	45	428	202	32

* Tubers inoculated 24. vi. 32.

† Tubers inoculated 4. vii. 32.

The results indicated that the better ventilation of the hampers was responsible for the reduction in disease. It was decided, therefore, to repeat the experiment, using ventilated barrels, since this is the chief package used in the trade. Barrels, with and without holes, were compared with hampers. In the first experiment the produce was sent to

Weymouth and returned to Jersey, a total sea journey of about ten hours, whereas in the second trial it was sent to Holyhead and back to Jersey, a total sea journey of about sixty hours. The results are given in Table V and show that ventilation of the barrels and hampers reduced the disease in the first experiment but not so much in the second.

Table V.
Relation of the package to disease.

Package	Ventilation	Exp. 1.* Tubers (19. vi. 33)			Exp. 2.† Tubers (29. vi. 33)		
		No. healthy	No. diseased	Diseased %	No. healthy	No. diseased	Diseased %
Barrel	None	488	250	34	315	310	50
"	"	611	145	19	351	304	46
"	48 holes (1 in. diam.)	638	101	14	437	165	27
"	"	755	78	9	350	300	46
"	24 holes (1 in. diam.)	621	85	12	383	264	41
"	"	624	72	10	345	167	33
Hamper	Normal	611	57	9	376	231	38
"	"	715	55	7	400	260	39

* Packages sent to Weymouth; tubers inoculated 9. vi. 33.

† Packages sent to Holyhead; tubers inoculated 20. vi. 33.

It is concluded that on a short sea voyage ventilation of the packages reduces blight in tubers contaminated with fungus spores; such ventilation is less effective on a longer sea voyage. Although there is no information on the point at present, it is possible that ventilation of the ship's hold would be necessary if ventilation of the packages is to have its maximum beneficial effect.

FUMIGATION OF PACKAGES.

The effect of introducing a fumigant into barrels when these were being packed for export was also tried. Following preliminary trials in the laboratory it was decided to use formaldehyde and flowers of sulphur. Freshly dug, healthy tubers were inoculated as described above and packed in barrels the sides of which had just been sprayed or dusted with the fumigant. The consignment was shipped to Weymouth and returned to Jersey. The results are given in Table VI and show that less disease occurred where formaldehyde was used. The test was repeated, using 100 c.c. of 40 per cent. formaldehyde and tubers dug from a diseased crop to obtain natural contamination. The treatment reduced the disease from 69 to 14 per cent., but unfortunately the tubers were spotted, owing, probably, to the use of too much formaldehyde.

Table VI.
Fumigation of packages.

No. of barrel	Treatment	Tubers		
		No. healthy	No. diseased	Diseased %
1	Sprayed with 100 c.c. water (control)	317	455	59
2	" 100 c.c. water (control)	317	403	56
3	" 100 c.c. water and dusted with 50 gm. sulphur	373	417	53
4	" 100 c.c. water and dusted with 50 gm. sulphur	365	376	51
5	" 30 c.c. formaldehyde (40 %) in 70 c.c. water	485	129	21
6	" 30 c.c. formaldehyde (40 %) in 70 c.c. water	579	131	18

The results indicate that fumigation may be useful in reducing disease in transit. It is not suggested that formaldehyde should be used; it is hoped that a more suitable fumigant will be found.

DISCUSSION AND SUGGESTED CONTROL METHODS.

The early potato crop in Jersey is one of the main sources of income for the grower. Unfortunately, owing to factors over which the farmer has little or no control, severe attacks of blight are liable to occur. One-third of the island is planted, which means that potato fields are often contiguous and that disease may spread with alarming rapidity. Blight is also favoured by the mild, humid climate, occasional sea-fogs, and by liberal manuring and close planting. The variety grown, International Kidney, is immune from wart disease (*Synchytrium endobioticum*), does not appear to suffer from virus diseases, and gives remarkably good yields although it has been cultivated in the island for more than sixty years; its only weakness is its susceptibility to blight. Search for a more resistant variety is proceeding, but there is little likelihood at present of one being found which will suit the island conditions so well as International Kidney. These considerations show that determined efforts are needed to produce a healthy potato crop in Jersey.

It has been seen that disease attacks the tubers in the field and in transit. Most of the latter is due to contamination of the potatoes at digging time by spores falling from blighted haulms. Such tubers spread disease to others in transit. The disease which appears early in the markets is usually caused in this way, and is especially harmful because it undermines the confidence of merchants for the rest of the season. Careful trials have shown conclusively that thorough and regular spraying will at least check the disease until the bulk of the produce has been exported, except, perhaps, in rare seasons when climatic conditions are very favourable for blight. Usually, spraying will keep the haulms

healthy until a satisfactory yield of tubers has formed. Once this stage has been reached farmers are urged to dig the crop at once or, if this is not possible, to cut or scorch the haulms while these are healthy or, at the latest, when the first sign of disease appears. It is believed that this combination of spraying and cutting or scorching the haulms will almost entirely eliminate not only the loss in transit but also that in the field.¹ While it may be possible in future to reduce the disease in transit by ventilation or fumigation of packages, such methods are intended for use in special cases only. The farmer should not rely upon these measures, but should check the disease at the source, *i.e.* on the growing crop in the field.

It is better to destroy the haulms as suggested above than to allow disease to do it, because once blight has become prevalent loss in the field is inevitable and loss in transit is difficult to avoid. The only way to reduce the latter is to allow sufficient time between the death of the haulms and digging so that infected tubers will be obviously diseased at harvesting time. The potatoes which are healthy when dug are likely to remain so later, since there are few or no spores present to cause contamination during digging.

SUMMARY.

1. In Jersey the loss due to potato blight (*Phytophthora infestans*) in crops grown for export may be divided into:

(a) Loss in transit caused by contamination of the tubers by spores at digging time and by packing tubers which are infected, but not obviously, when dug.

(b) Loss in the field, which includes diseased potatoes left on the ground at harvesting time.

2. These losses, excepting those caused by contamination, occur when the disease on the haulms has reached an advanced stage before harvesting. The remainder occurs when disease is active on the haulms at digging time.

3. It is shown that blight may spread from diseased or contaminated tubers during transit and that there is little or no danger in using barrels which have previously contained diseased produce.

4. Ventilation or fumigation of the packages reduced the disease in transit.

5. Control methods are suggested and discussed.

¹ Since this was written, scorching the haulms with sulphuric acid (B.O.V., s.g. 1.7) has been tested and has proved very effective on the green haulms in Jersey. Three to four gallons of acid in 40 gallons of water are sufficient.

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FUSARIUM SPECIES ON BRITISH CEREALS

BY F. T. BENNETT, B.Sc., PH.D. (LOND.).

(Advisory Mycologist, Armstrong College, University of
Durham, Newcastle-upon-Tyne.)

(With Plates XXI and XXII and 9 Text-figures.)

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INTRODUCTION.

CERTAIN species of *Fusarium*, which are more or less pathogenic toward cereals in Great Britain, have been described by the writer in previous volumes of these *Annals*.¹ When investigating cases of plant or crop failure, however, species other than those described are frequently found, alone or in association with the former, or with such a pathogen as *Ophiobolus graminis*. Nearly thirty species of *Fusarium* have now been isolated from British cereals; not all of these have yet been investigated in detail, but of those investigated some have proved to be pathogenic and others merely saprophytic. It is obvious that the presence of some *Fusarium*, other pathogens being absent, is not sufficient evidence that the *Fusarium* is the primary cause of defective growth in any given

¹ These species, arranged in sections as above, are: Section Roseum: *F. herbarum* (Cda.) Fr. *var. avenaceum* (1a). Section Gibbosum: *F. Scirpi* (Lamb. et Fautr.) (1b). Section Discolor: *F. graminearum* Schwabe = *Gibberella Saubinetii* (Mont.) Sacc. (1c), *F. culmorum* (W. G. Sm.) Sacc. (1a). Section Arachnites: *F. nivale* (Fr.) Ces. (1d).

plant or crop. From a phytopathological point of view it is essential that the species of *Fusarium* be identified, and to have information as to the pathogenicity or otherwise of that species. For this purpose the general mycologist requires easily accessible, concise descriptions and typical illustrations, with an account of the simplest cultural work that will suffice for the identification of species. The only collective publication for the diagnosis of species is Wollenweber's *Fusarium Monographie* (7d), invaluable to the specialist but scarcely satisfactory for the general worker, and unfortunately not including the Sections *Roseum* and *Sporotrichioides*. There is a feeling of doubt amongst mycologists as to the possibility of identifying species, a doubt arising from certain developments in studies of *Fusarium* by various investigators. Wollenweber (7), Butler (3a), Brown (2), Leonian (4) and others, have given ample evidence that by certain cultural technique many species of *Fusarium* can be greatly modified, and even rendered indistinguishable from one another. This was followed by Mitter's (5) statement that the specific characters are not distinctive and the sectional grouping of species has no satisfactory basis. The contrary view is that there is abundant evidence of morphologically good species in nature, and that the morphological characters of the majority of individual growths from naturally produced spores *do* furnish an adequate conception of a species unit. This latter view is undoubtedly correct within the writer's experience, and is the basis of the monograph mentioned; mycologists may proceed confidently with the identification of the species of common occurrence on British cereals.

Comparatively few species of *Fusarium* can be identified with certainty from their occurrence under natural conditions where the diversity of environments modifies considerably, though temporarily, the morphological characters; restoration of specific characters with uniformity is effected by simple cultural work, but due allowance must always be made for such amount of variation in cultures as is inherent within the orbit of a species. Repeated subculturing and special technique will generally result in degeneration or the introduction of variants, which, although unnatural, may remain permanent or dominant in cultures. In other words, first cultures from natural sources are of the greatest diagnostic value, and a number of these should be considered together to get a comprehensive view of the specific characters as regards the occurrence, or special forms, of microconidia, sclerotia, chlamydospores, etc., which frequently disappear in subsequent subcultures. Incidentally, type cultures of *Fusarium* generally require interpretation in

the light of these remarks, otherwise they may be quite misleading. The final factor in the diagnosis of a species is the size and shape of macroconidia; other features, though often affording valuable guidance, are definitely subsidiary. It has been remarked of the illustrations of macroconidia in this paper that there is no great difference between some of them; differences there are, however, and to exaggerate them or to illustrate a single type form would not equally assist investigations. The illustrations throughout the paper are on a uniform scale to facilitate comparisons, and the descriptions given, except where otherwise stated, are for the first growths of single conidia on wheat-meal agar. Whilst wheat-meal agar¹ is the simplest and most generally useful artificial medium for first-culture work, it should be emphasised that no one medium, natural or artificial, suffices to induce distinctive characteristic features for all species. Consequently in this paper reference is made to other media also, especially those bringing out characteristics useful for purposes of verification.

SECTION ROSEUM.

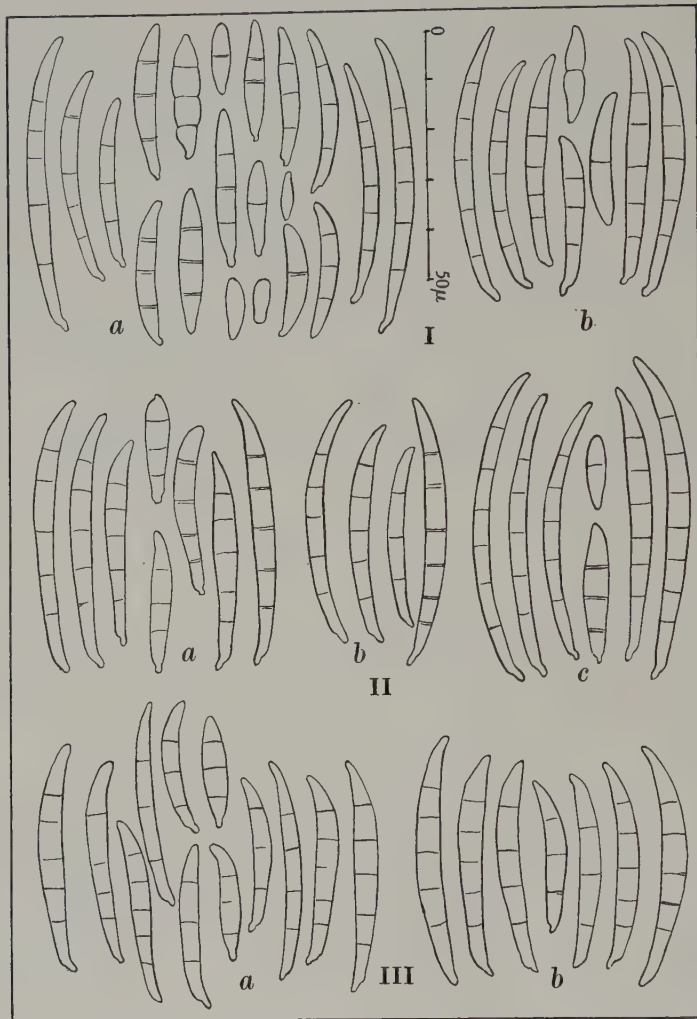
F. herbarum (Cda.) Fr.

F. herbarum (Text-fig. 1) is one of the commonest species occurring on agricultural and horticultural crop plants, particularly on those unhealthy, dying, or dead. Because the species shows considerable range of characters under natural conditions, and to less extent in artificial cultures, it has been variously named by different observers; for the many synonyms and varieties see *Fusarium Monographie* (17d), pp. 437–76). On cereals in the field it often occurs as a thin film of spores of pale orange colour in the furrows of nodes on the straw and along the edges of glumes. It is common on the aerial and underground parts of grasses also, and persists in vegetable residues in the soil. On incubation, infected material gives slight to obvious aerial mycelium with few microconidia, and later some macroconidia also; small sporodochia consisting of spores of mixed types develop slowly on the material and on the paper around it.

Characteristics of single-conidium cultures on wheat-meal agar.

Aerial mycelium. Abundant, floccose, white with usually rosy tints. At 1 month the basal mat carmine or mixed carmine and ochraceous colours, and the superficial growth somewhat matted. Blue-green coloration, from mere dots to patches 4 mm.

¹ "Whole" wheat meal 60 gm. stirred in 1000 c.c. of cold tap water, warmed in a water-bath to 60° C. for 15 min., 15 gm. agar added, steamed until dissolved, strained through fourfold cheese-cloth, made up to 1000 c.c. Tubed portions steamed for 20 min. on three successive days. For other media see *Ann. Appl. Biol.* xvii, 48, 1930. Ridgway's Colour Standards used for colour references.

Text-fig. 1. *Fusarium herbarum* (Cda.) Fr.

- I. From wheat-meal agar; (a) microconidia from aerial mycelium of culture 4 weeks old; (b) macroconidia from sporodochia of culture 6 weeks old.
- II. From hard oat agar; (a) sporodochial, culture 2 months old; (b) and (c) sporodochial, cultures 4 months old, (c) showing the more lanceolate forms resembling those of var. *avenaceum*.
- III. From cooked potato; sporodochial, cultures 2 months old; (a) some of the varied forms; (b) typical forms predominating.

in diameter, frequent in first cultures on the thin film of medium above the slant.

Substratum. Plectenchymatic layer pomegranate purple to carmine as a whole; or in the basal part only, when the upper parts shade through Eugenia or Pompeian red to nil. At 2 months the plectenchyma is Bordeaux purple or ox-blood red, or these colours around the base and shading to near brown in the upper parts.

*Stromata and sclerotia.*¹ Plectenchymatic aggregates generally numerous on the film of medium above the slant, along the edges, at the back of the medium about the apex, and on the surface of matted mycelium. Commonly 1 mm., but many 3 mm. in cultures 2 months old; occasionally those on the mycelium increase to 5 mm. diameter, 2 mm. high, and approach stipitate form. Generally honey yellow to golden brown, but occasionally greenish blue basally and internally.

Sporodochia. Arise from the plectenchymatic surface, pushing through the mycelial mat in tubercular clusters, commonly 3 mm. in diameter, but sometimes up to 7 mm. across and 3 mm. high; the size increases over many months whilst moisture remains in the medium. Colour depends to some extent upon exposure to light; apricot orange, salmon orange, or bittersweet pink at first, fading with age (2-3 months) towards rufous. No pseudopionnotal form has been observed.

Conidia. (a) On aerial mycelium from rare to numerous, of extremely varied forms ranging through oblong, spindle and sickle shapes; the majority are sickle-shaped and 1- or 3-septate, with a few 5-septate.

(b) In sporodochia two main types; one rather short and broad, the other more slender with considerable resemblance to macroconidia of variety *avenaceum*. There is so much variation that extremes are better left out of consideration in favour of the more numerous typical forms. Walls and septa are very delicate:

(1) From natural film on wheat culms: 3-septate, 70 per cent., 15-37.5 × 2.5-4.0; average 29 × 2.9 μ . 0- and 1-septate, 30 per cent.

(2) From sporodochia on wheat-meal agar, culture 6 weeks old: 3-septate, 5-10 per cent.; average 31.5 × 3.35 μ . 5-septate, 90-95 per cent., 39-60 × 3.25-4.85; average 48.5 × 3.55 μ .

(3) From sporodochia on hard oat agar, in which the two types are more pronounced; the septation mode, and the ratio length to breadth vary as cultures age.

Septation	At 1 month		At 2 months		At 3 months	
	%	Size (μ)	%	Size (μ)	%	Size (μ)
3-	27	36.0 × 4.0	—	—	—	—
4-	10	40.0 × 4.5	12	49.0 × 3.5	—	—
5-	63	(a) 45.0 × 5.0 (b) 50.0 × 5.0	58	(a) 50.0 × 4.8 (b) 54.0 × 4.1	60	(a) 53.1 × 4.8 (b) 57.5 × 3.4
6-	Rare	—	27	(a) 65.0 × 4.3	30	(a) 61.1 × 4.75 (b) 78.0 × 3.0
7-	—	—	3	(a) 70.0 × 4.3	10	(a) 61.2 × 4.8 (b) 78.2 × 3.0
8-	—	—	—	—	Rare	(a) 62.5 × 5.0 (b) As 6- and 7-

(a) = broad, (b) = slender type, from the same sporodochium.

Other media. Cooked potato favours the production of patches of blue-green colour in the aerial mycelium; large (up to 5 mm.) plectenchymatic stromata often

¹ See footnote, p. 495.

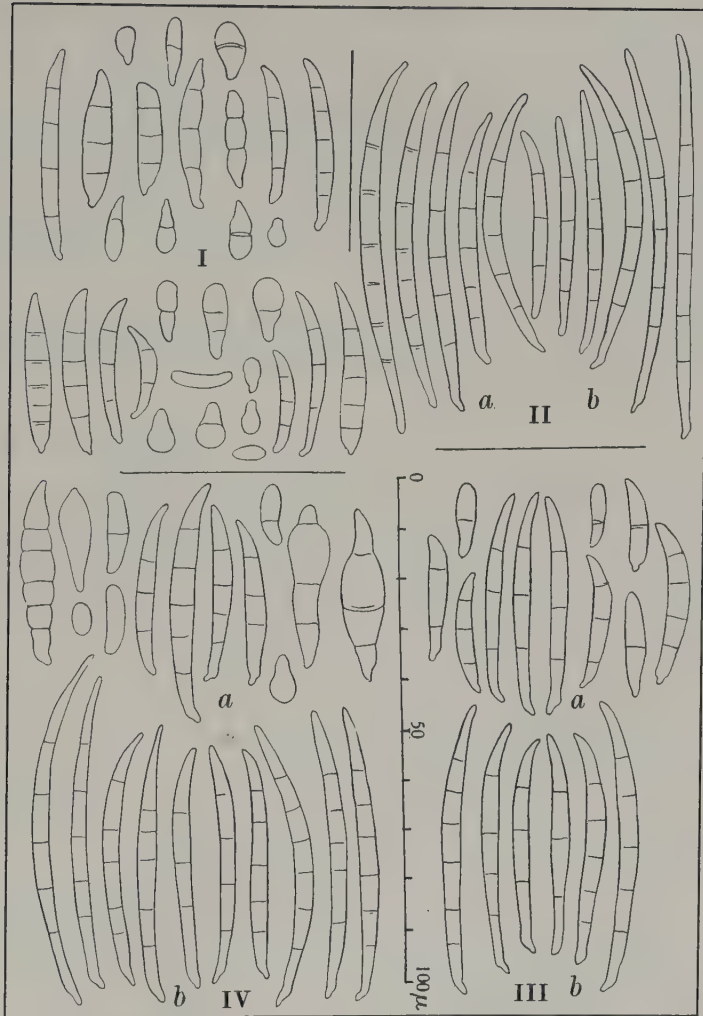
of blue colour basally; sporodochia are few, large, and of salmon orange colour. Synthetic media at pH 5.5; with dextrose gives carmine plectenchyma with lilac to plum shades below; with glycerine stromata are numerous but small, and of greenish blue to light blue colour.

Chief cultural characters. Aerial mycelium; well developed, white with rosy tints. Plectenchymatic layer; carmine, pomegranate or Bordeaux purple. Stromata and sclerotia; from small (0.5–1 mm.) to large (5 mm.), honey yellow, sometimes blue basally. Sporodochia usually large (5 mm.), tuberculate, salmon orange coloured; the macroconidia in any sporodochium not uniform; typically 5-septate, 3.5–4.5 μ wide; ratio length to breadth 11.3 to 1. Microconidia usually rare, none pyriform. Chlamydospores, none.

Pathogenicity. Wheat grown on sand under abnormally moist and warm conditions, the seed inoculated by contact with pure cultures of *F. herbarum*, yielded 96 per cent. of affected seedlings; seed immersed in an aqueous suspension of spores gave 85 per cent. of affected seedlings. Artificially contaminated seed grown in clean soil, and clean seed grown in contaminated soil, yielded in each case 100 per cent. of affected plants under normal conditions of moisture and temperature. The fungus was recovered from basal parts, and from the stem internode above soil level in 90 per cent. of the plants from contaminated soil, and 75 per cent. of those from contaminated seed. Affected plants showed discoloration of the basal parts and browning of roots, but were not killed off. Under normal conditions growth was continued throughout the season, the plants being paler in colour than controls, leaves generally yellow tipped, straw shorter and grain lighter. It was concluded that *F. herbarum*, whether occurring on the seed or in the soil, would under abnormal conditions cause seedling blight, and under normal conditions a mild but persistent foot rot resulting in reduced yield of straw and grain. In the latter case the occurrence of disease would not be recognised by a grower.

F. herbarum (Cda.) Fr. *forma* 2 Wr.

This fungus (Text-fig. 2), formerly *F. herbarum* (Cda.) Fr. var. *gibberelloides* Wr., is described by Wollenweber (7c) as follows: "A typo differt sclerotiis numerosis magnis globosis v. rugulosis atrocaeruleis Gibberellae similibus. Hab. in rimis corticis Robiniae pseudacaciae, Dahlem." The organism as isolated from leaves and bases of wheat by the present writer was kindly identified by Dr Wollenweber. Although this fungus is not common on cereals, its recognition is desirable because its characters resemble in part other fungi that are common. Infected material on incubation yields obvious woolly to floccose aerial mycelium bearing microconidia of pyriform, oblong, reniform, allantoid and falcate



Text-fig. 2. *Fusarium herbarum* (Cda.) Fr. *forma* 2 Wr.

- I. Microconidia from aerial mycelium, cultures 6 weeks old; above from wheat-meal agar, below from hard oat agar.
- II. From wheat-meal agar, cultures 2 months old; (a) and (b) different sporodochia.
- III. From single-conidium subcultures, 2 months old, on wheat-meal agar; (a) microconidia from aerial mycelium, pyriform rare to absent; (b) macroconidia, sporodochial, now shorter type.
- IV. From mass-inoculum subcultures, 2 months old, on cooked potato; (a) microconidia from aerial mycelium; (b) macroconidia, sporodochial.

shapes; these conidia are not distinctive, but the prevalence of pyriform ones is notable.

Characteristics of single-conidium cultures on wheat-meal agar.

Aerial mycelium. Well developed, floccose, white with tints from pomegranate purple to vinaceous, especially near the substratum; occasionally patches tinted Medici blue.

Substratum. Plectenchymatic layer amaranth, pomegranate, to Bordeaux purple, with deeper parts eventually greyish brown; in subcultures the colour may be reduced to Eugenia red.

Stromata and sclerotia. Plectenchymatic nodules, commonly 2-3 mm. high but sometimes up to 5 mm., with tuberculate surface, of honey yellow colour; occasional ones with tips of tubercles of blue-green colour. Sometimes also small sclerotia, 1 mm. across, blue-green, pimple- or disc-like.

Sporodochia. Appear in from 3 to 4 weeks; apricot to orange buff, subsequently rufous to ferruginous; singly, 1 mm., but generally in small clusters on decumbent mycelium, on small sclerotial discs, or on tubercles of larger stromata.

Conidia. (a) Microconidia on aerial mycelium, especially about apex of slants, and on or around large stromata, pyriform, oblong, reniform, allantoid and falcate.

(b) Macroconidia in sporodochia, 40 to 93 per cent. 5-septate, very slender, curved, with long apical cell, and definite pedicel; walls and septa exceedingly delicate.

(1) Sporodochial, on wheat-meal agar, culture 2 months old; proportion of 5-septate 40-93 per cent. in sporodochia of different cultures.

(a) 3-septate, 2 per cent., 4-septate, 5 per cent.; average $41.6 \times 2.7 \mu$. 5-septate, 93 per cent.; average 52.3×3.5 ($49.5-57.5 \times 2.9-3.9$) μ .

(b) 3-septate, 30 per cent., 4-septate, 30 per cent.; average $43.9 \times 2.9 \mu$. 5-septate, 40 per cent.; average 51.9×3.3 ($41.5-62.4 \times 2.7-3.9$) μ .

(2) Sporodochial, on wheat-meal agar, culture 3 months old; 3- and 4-septate, 5-10 per cent.; 5-septate, 60-70 per cent.; average 61.0×2.7 ($52-75 \times 2.5-2.8$) μ . 6-septate, 25-30 per cent.; average 70×2.75 ($52-75 \times 2.5-2.8$) μ .

(3) Sporodochial, from six kinds of substrata; 3- and 4-septate comparatively rare. 5-septate, from 35 per cent. on maize-meal agar at 3 months to 95 per cent. on sterile wheat straw; average 58.4×3.04 ($41.5-80.5 \times 2.5-3.9$) μ . 6-septate, from 2 per cent. on sterile wheat straw to 40 per cent. on some hard oat agar cultures 3 months old; average 65.8×3.16 ($50-78 \times 2.7-3.9$) μ .

Other media. Cooked potato bears a dense aerial mycelium, white with vinaceous tints and occasionally bluish patches; the substratum pomegranate to Bordeaux purple, sometimes ivy green at the upper end. Stromata are large, often 3-4 mm. in diameter and 2-3 mm. high, tuberculate, and olive lake to greenish blue superficially. Microconidia from around the stromata include enlarged pyriform and spindle-form ones amongst the more numerous falcate shapes. Macroconidia in sporodochia are 5-septate up to 95 per cent.

Hard oat agar also favours distinctive features; stromata up to 5 mm. high, and abundant microconidia; sporodochial macroconidia are of almost acicular form, with apical cell $15-20 \mu$ long, 5-septate up to 70 per cent., $52-80 \times 2.6-3.0 \mu$.

Sterile wheat straw inoculated from aerial mycelium of first cultures bears sporo-

dochia of very uniform conidia, 5-septate up to 95 per cent.; average 67.6×2.63 ($45.5-78 \times 2.5-3.4$) μ .

Notes. The species changes rapidly in subsequent subcultures according to the kind of inoculum transferred; single conidia yielded after prolonged artificial culturing also give more or less abnormal growth forms.

Chief cultural characters. Aerial mycelium well developed, white with purple to vinaceous tints, and occasional blue patches. Plectenchymatic layer pomegranate to Bordeaux purple. Sporodochia usually 1 mm., but sometimes massed; no pseudopionnotal form; macroconidia typically 5-septate, $2.5-3.9 \mu$ wide, and ratio length to breadth 19.3 to 1. Stromata commonly up to 5 mm., tuberculate, tubercles blue-tipped or not, and either bearing sporodochia or not. Microconidia, pyriform ones noticeable amongst the varied forms on aerial mycelium. Chlamydospores, none.

Pathogenicity. Not yet investigated.

SECTION GIBBOSUM.

F. Equiseti (Cda.) Sacc.

This fungus (Text-fig. 3, Plate XXI), under the name of *F. falcatum*, has been fully described by Wollenweber (7a), p. 184, and the diagnosis is in *Fusarium Monographie* (p. 330); it is included here in accordance with the writer's plan for identification of cereal-inhabiting species of *Fusarium*. It has been recorded from *Equisetum*, *Asparagus*, *Callistephus*, *Lycopersicum*, *Hordei*, *Triticum*, *Lupinus*, *Pisum*, *Phaseolus*, *Sambucus*, *Solanum*, humus, etc. The present writer has found it associated with the basal parts of wheat and barley showing foot rot in the field. On incubated infected material it appears as a very sparse web-like growth between adjacent roots and minute cream-coloured sporodochia on the roots themselves. The fungus has not been seen on aerial parts in nature, but probably so exists as it is known to attack maturing grain under experimental conditions.

Characteristics of single-conidium cultures on wheat-meal agar.

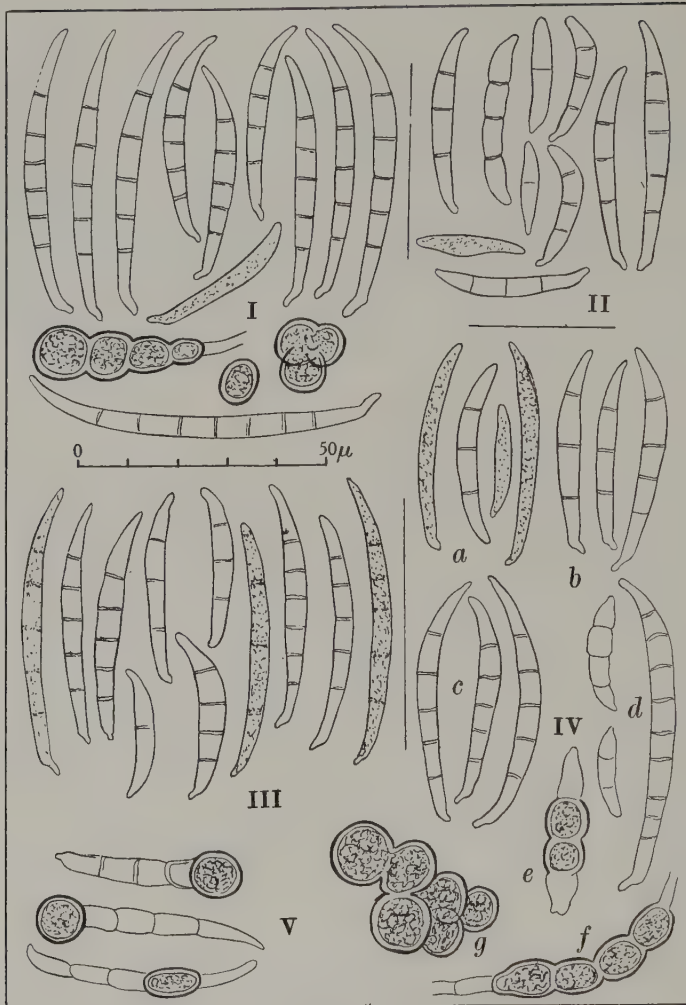
Aerial mycelium. Sparse web-like hyphae masked by sporodochial deposits thereon; a small arc of white mycelium persists at the top of slants. After some months a very thin, white hyphal growth covers the pseudopionnotes on the lower part of slants.

Substratum. Not coloured except as and by the submerged plectenchymatic mycelial layer; eventually the deeper parts about clay colour.

Pseudopionnotes. Covering the surface in from 2 to 3 weeks; buff pink, light ochraceous salmon, or light pinkish cinnamon; after about 6 weeks light pinkish cinnamon to light vinaceous cinnamon, and subsequently light clay colour.

Conidia. (a) Microconidia occur in the sparse mycelium at the tops of slants; variable in form, more or less parabolic curvature, 3-septate up to 75 per cent.

(b) The characteristic features of macroconidia are the parabolic to elliptical dorsal curvature, apical cell longer than others and tapering uniformly, basal cell sometimes

Text-fig. 3. *Fusarium Equiseti* (Cda.) Sacc.

- I. From seedling wheat incubated 4 weeks; conidia from web-like growth between roots, chlamydospores from along stems.
- II. From wheat-meal agar; microconidia from sparse aerial mycelium at tip of slant culture.
- III. From wheat-meal agar, pseudopionnotal, culture 4 weeks old.
- IV. From salts-glycerine agar; (a) and (b) 0- to 4-septate forms; (c) typical 5-septate; (d) miscellaneous; (e), (f), (g) chlamydospores.
- V. Chlamydospores, conidial, from cultures on wheat straw.

long also and with well developed pedicel, slender appearance, exceedingly thin walls and equidistant septa.

(1) Pseudopionnotal, various cultures, 1 month old; 0-, 1-, 2-septate, present but few. 4-septate, 10 per cent. 3-septate, 30-50 per cent.; average 34.8×3.2 ($25-45 \times 2.9-4.5$) μ . 5-septate, 40-60 per cent.; average 44.5×3.75 ($36-57 \times 3.25-4.5$) μ . 6-septate, occasional; $52 \times 4.1-4.2$ μ .

(2) Pseudopionnotal from six kinds of substrata; septation mode varies greatly according to the substratum, thus: 3-septate, 75 per cent. on straw, 10 per cent. on cooked potato; 5-septate, 5 per cent. on straw to 75 per cent. on cooked potato; 6-septate, from 0 to 1 per cent. on both substrata. Average sizes are: 3-septate, 36.8×3.6 μ ; 5-septate, 48.8×4.3 μ ; 6-septate, 57.0×4.6 μ ; 7-septate, rare, 66.1×5.0 μ .

Chlamydospores. In wheat-meal agar cultures rare or none until the superficial hyphal layer develops on the pseudopionnotes, and similarly for most other artificial media; then in hyphae, singly, in short chains, or small clusters. Fairly numerous on the surface of incubated infected natural material, and in the hyphal tuft at the top of cultures on straw. Conidial chlamydospores rare, nearly always single and terminal, occurring in old cultures on straw, hard potato agar, and other substrata of low nutrient value. 7-14 μ in diameter, and of pale cinnamon tint.

Other media. Infected natural material, such as wheat bases, yields sporodochial conidia with more distinctive and uniform curvature and a higher septation mode: 0-, 3-, 4-septate up to 5 per cent. 5-septate, 95 per cent.; average 49.9×4.5 ($41.6-65.0 \times 3.5-5.1$) μ . 6-septate, 1 per cent.; average 62.4×4.8 ($59-65 \times 4.0-5.0$) μ . 7-septate, occasional; average 66.0×5.0 ($59-72 \times 4.9-5.1$) μ . Chlamydospores after 1 month, up to 10 μ diameter, pale cinnamon.

Sterile wheat straw bears, after about 1 month, minute sporodochia; the 5-septate conidia are more typical and uniform than in artificial cultures on any other substratum. 3-septate, 75 per cent.; average of normally shaped 37×3.3 μ . 4-septate, 10 per cent. 5-septate, 5 per cent.; average 48×4.0 ($40-50 \times 3.5-4.5$) μ . 6-, 7-septate, 3 per cent.; average 49.5×4.5 ($47-52 \times 4.0-4.6$) μ . Chlamydospores occur in conidia, surface hyphae and the apical tuft of mycelium; younger forms hyaline, larger and older ones of cinnamon colour, 5-10 μ in diameter.

Notes. No definite and permanent mycelial form of this fungus has been obtained under any conditions; the mycelial forms of allied fungi are described for *F. Equiseti* f. 1, on p. 492, and for *F. Scirpi* in a previous publication (1b). Potato agar with 5 per cent. of dextrose bears over the pseudopionnotes a thin mycelial layer of rapidly germinating conidia, but this does not correspond to the mycelial forms mentioned. The hyperbolic dorsal curvature of conidia, characteristic of species in group *Gibbosum*, is rarely well marked in artificial cultures of *F. Equiseti*.

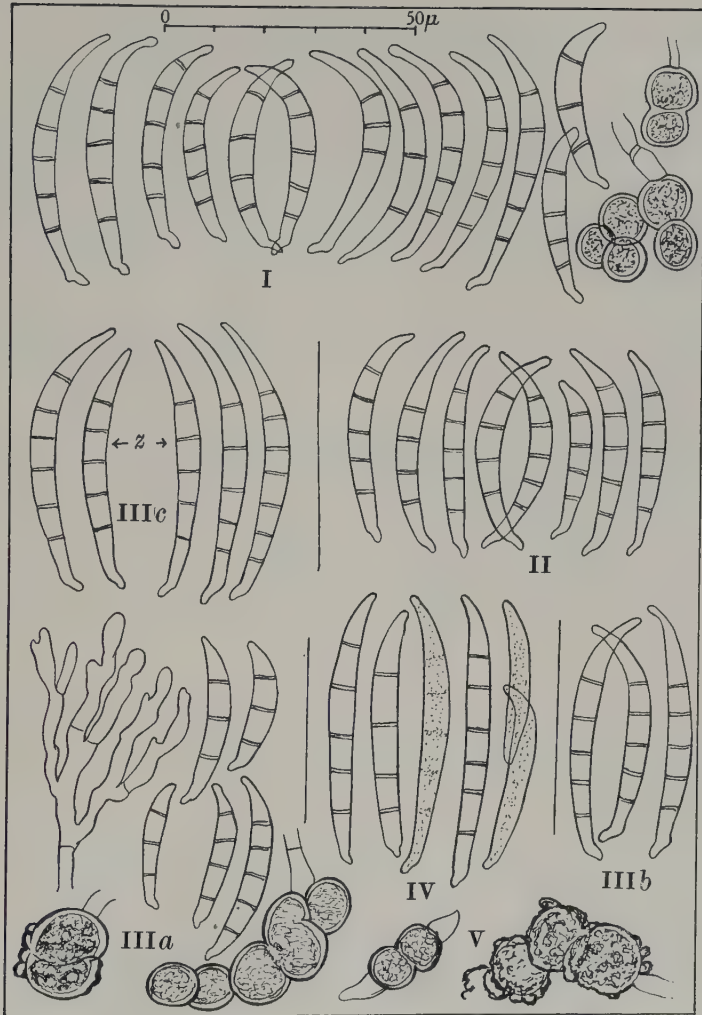
Chief cultural characters. Single-conidium cultures yield the pseudopionnotal form of growth; little or no aerial mycelium except a thin layer from germinating conidia. The pseudopionnotes is at first of ochraceous cream colour, and may remain so with little darkening. Conidia mainly 5-septate, with extremely delicate walls and septa, and some with characteristic curvature. Chlamydospores in hyphae, tinted cinnamon.

Pathogenicity. Wheat, barley and oats, grown in contaminated soil or from contaminated seed in clean soil, under normal conditions of

moisture and temperature are attacked by *F. Equiseti*. The fungus extends readily up the first sheathing leaf, and frequently penetrates the stem internode immediately above soil level. There is little or no "seedling blight" in the sense of death of seedlings, but infected seedlings are thinner in the shoots, paler in leaf, and of poorer growth generally. This incipient foot rot persists throughout the growth of the plants, causing the production of more abundant, but useless, tillers, and although the ear-bearing stems may be as numerous as in healthy plants, the straw is thinner and shorter, and grains fewer and smaller. The ear-blight stage is equally obscure. Both wheat and barley are attacked, more especially the older ears and at the higher summer temperatures. Under experimental conditions the glumes are discoloured with bleached and brownish diffuse patches, wheat grains are of dingy brown colour and barley grains brownish to greenish grey. Germination of grains from ears inoculated during growth was reduced by from 25 to 75 per cent. Choosing from the germination tests the viable grains only, and ensuring their freedom externally from fungus, many still gave poorer seedlings, indicating that the fungus is conveyed internally in the seed also. Under outdoor conditions spore production is restricted, and casual infection by wind-borne spores from an infection centre is also restricted in cereal crops; hence this fungus is less common than some other species of *Fusarium*. Further, the foot-rot and ear-blight phases are easily overlooked, and the damage done is more likely to be attributed by ordinary observation to poor seed, soil or season than to fungal disease.

F. Equiseti (Cda.) Sacc. *forma* 1 Wr.

This fungus (Text-fig. 4, Plate XXII), is referred to in comparatively recent literature as *F. ossicolum* (Berk. et Curt.) Sacc., and *F. falcatum* App. et Wr. var. *fuscum* Sherb. Other synonyms and a brief diagnosis are given in *Fusarium Monographie*, p. 330. The fungus has been recorded from Cucurbitaceae, Aesculi, Asparagi, Atriplex, Clematidis, Gossypii, Zeae, Lycopersici, Solani, Typhae, animal bones and humus. The present writer has found it of frequent occurrence on underground and aerial parts of wheat, barley and oats in Britain. From natural sources and in artificial cultures it differs considerably from the type species, although certain characteristics in common have led to the present nomenclature. Naturally infected material, such as the basal parts of cereal plants, on incubation gives scanty mycelial growth with minute, pale ochraceous sporodochia on the hyphae or apparently on the substrata; there is no abundant mycelium as in first cultures. The sporodochial conidia here



Text-fig. 4. *Fusarium Equiseti* (Cda.) Sacc. forma 1 Wr.

- I. From barley roots after incubation 14 days.
- II. From wheat seedlings after incubation 4 weeks.
- III. From wheat-meal agar; (a) from aerial mycelium, and (b) from thin film of medium, at 4 weeks; (c) from sporodochia formed when cultures 3 months old, (z) dominant.
- IV. From wheat-meal agar; pseudopionnotal, from subculture showing loss of characteristic form.
- V. Chlamydospores; warted ones from first cultures only.

produced generally show marked hyperbolic curvature, the apical cell short with bulbous termination, and well-developed pedicel.

Characteristics of single-conidium cultures on wheat-meal agar.

Aerial mycelium. Abundant, at first white, but within 2-3 weeks tinted brownish above and cinnamon next the substratum; at 4 weeks matting down, cinnamon above to tawny below. The shades are due mainly to chlamydospores—first an increase in number and later development of cinnamon colour.

Substratum. Plectenchymatic layer Dresden brown, hazel, to chestnut brown; below this layer the medium passes through light cinnamon, dark hazel, to cinnamon or warm sepia.

Stromata. Plectenchymatic humps, 1-2mm. in diameter, honey yellow or brownish-occasional in first cultures; an increase in number and size can be produced in subcultures by appropriate methods.

Sporodochia. Few, up to 3 mm. in diameter, developing slowly—appearing usually in from 2 to 3 months, ochraceous tawny. Smaller sporodochia and incomplete pseudopionnotes on the surface below the mycelium can be produced in subcultures more speedily by appropriate methods.

Conidia. (a) Microconidia occur on aerial mycelium; these are commonly 3- and 5-septate, and resemble macroconidia more or less.

(b) Macroconidia have strongly defined walls and septa, well developed pedicel, apical cell either elongate and sharply bent or shorter and having a small bulbous apex, and generally 5-septa; well-marked parabolic dorsal curvature is usual in first cultures.

(1) Sporodochial, culture 4 months old. 5-septate, 70 per cent.; average 50.3×4.95 ($41-57 \times 4.1-5.2$) μ . 6-septate, 29 per cent.; average 53.8×5.1 ($46-60 \times 4.9-5.2$) μ . 7-septate, 1 per cent.; average as 6-septate.

(2) Sporodochial, subculture 15 days old; septation indistinct, average 47.5×4.8 ($30-50 \times 4.0-5.1$) μ .

(3) From film of medium above slants, cultures 1 month old. 3-septate, 2 per cent.; 4-septate, 10 per cent. 5-septate, 75 per cent.; average 48.6×5.1 ($39-57.5 \times 4.9-5.8$) μ . 6-septate, 2 per cent.; average 52.0×5.2 ($45-65 \times 5.0-6.0$) μ . 7-septate, 1 per cent.; average 59.0×5.2 (range as 6-septate) μ .

(4) Macroconidia from various artificial substrata. 5-septate, average 48.0×5.0 μ . 6-septate, average 53.7×5.1 μ . 7-septate, rare, average 58.0×5.15 μ .

(5) Macroconidia from incubated natural material. 5-septate, average 47.0×5.05 μ .

Chlamydospores. Abundant in mycelium, singly, in chains or clusters; 10-17 μ , average 15 μ in diameter; soon tinted, and later cinnamon coloured. Chlamydospores in first cultures are often "warted" when 2-3 months old, notably on potato dextrose agar and to less extent on hard oat, salts glycerine, and wheat-meal agar media. This peculiarity has not been observed in subsequent subcultures. Conidial chlamydospores are intercalary, occasionally terminal, and usually single; they occur in cultures from 1 to 2 months old on most media, but are most common on salts glycerine agar pH 6.6.

Other media. Hard potato agar bears conidia with septation ranging from 0 to 11, and length up to 78 μ in cultures 4-6 weeks old. The mycelium is scarcely tinted, and

chlamydospores faintly so, though very numerous, and frequently "warted". Sterile wheat straw used for subcultures bears small ochraceous sporodochia of particularly uniform, 5-septate conidia.

Notes. Considerable changes occur in the shape of conidia by repeated subculturing. The mycelial form of growth is maintained, but with reduction of chlamydospores, by subculturing from mycelial sources. In subcultures derived from mass inoculum containing a high proportion of macroconidia, the aerial mycelium is greatly reduced and sporodochia are small and numerous, often forming pseudopionnotal patches. Intermediate forms are common.

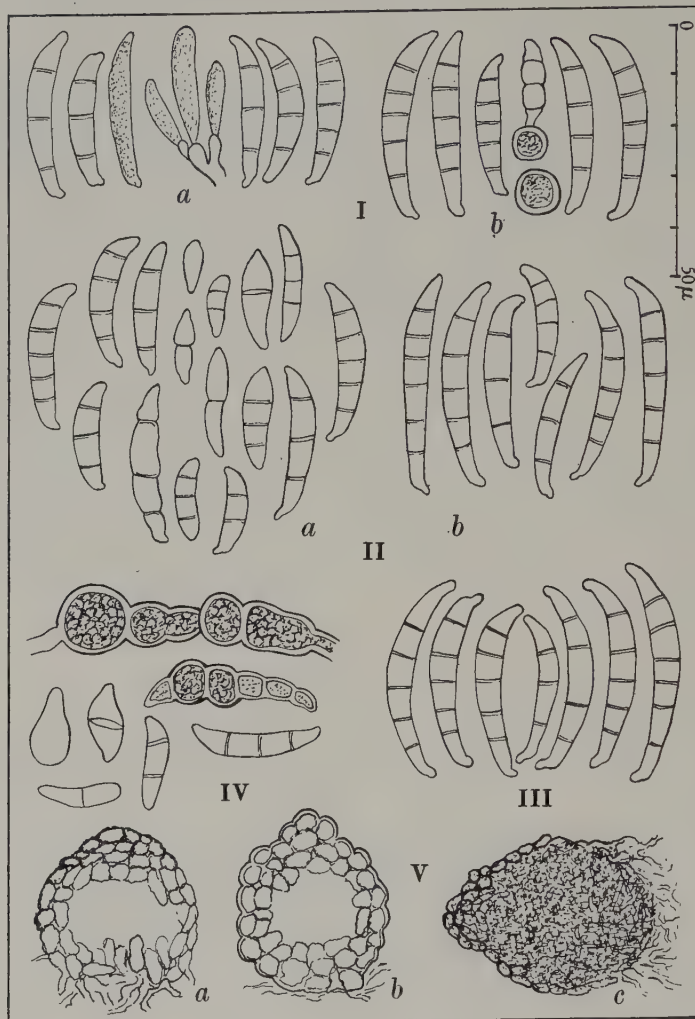
Chief cultural characters. Aerial mycelium abundant, dense, and cinnamon tinted in from 2 to 3 weeks. Chlamydospores abundant in the mycelium, cinnamon coloured, and frequently "warted". Sporodochia single and large, but of very slow growth. Macroconidia mainly 5-septate, with parabolic dorsal curvature, and strongly defined walls and septa.

Pathogenicity. The remarks made under *F. Equiseti* apply equally to this species form, except that in moist weather the lesions on ears of wheat and barley are here more obvious, and the fungus itself develops mycelial tufts at the tips of glumes or bases of awns. Whilst not destructive as a seedling blight or foot rot organism, it can, and does, cause considerable loss by reduction of germination capacity and yield of grain.

SECTION DISCOLOR.

F. Sambucinum Fuckel.

The numerous synonyms of this fungus (Text-fig. 5) are given in *Fusarium Monographie* (7d), p. 352), those of more interest being *F. discolor* App. et Wr., and *F. discolor* App. et Wr. var. *triseptatum* Sherb. Sherbakoff(6) states that his isolants differed markedly from *F. discolor* mainly by dominance of 3-septate conidia, larger plectenchymatic stromata, and larger sporodochia, adding that in certain instances the two organisms approach each other so closely as to be distinguished with considerable difficulty if at all. Wollenweber includes the type and the variety in one species. It will be apparent that the organism may show considerable differences when isolated from different substrata and under different conditions of environment. It has been recorded from roots, stems, leaves and fruits of numerous herbaceous plants, shrubs and trees, and from *Avena* and *Zea*. The present writer has found it on the basal parts of wheat, oats, and perennial rye-grass, on viola cuttings, and on roots of chrysanthemum. Infected material, on incubation, yields an aerial mycelium, white with tints of salmon or pale cinnamon colour, bearing a few microconidia, and in from 10 to 14 days small, ochraceous-cream tinted sporodochia of 3- to 5-septate macroconidia.



Text-fig. 5. *Fusarium Sambucinum* Fuckel. = *Gibberella pulicaris* (Fr.) Sacc.

- I. From natural material; sporodochial after (a) 10 days, (b) 30 days' incubation.
- II. From wheat-meal agar; (a) microconidia from aerial mycelium of cultures from 3 weeks to 3 months old; (b) macroconidia, sporodochial, cultures 3 weeks old.
- III. From wheat-meal agar; sporodochial, culture 3 months old.
- IV. From maize-meal agar; culture 2 months old; conidial and hyphal chlamydospores, 3-septate conidia dominant and pyriform ones reappear.
- V. Perithecial structures, three stages of development; not to scale—see text.

Characteristics of single-conidium cultures on wheat-meal agar.

Aerial mycelium. Well developed, compact and woolly next the substratum, open and floccose superficially; white, with slight tints of salmon to pinkish cinnamon in 3 weeks.

Substratum. Plectenchymatic layer salmon pink, or ranging from flesh to Japan rose; at 2 months pale pinkish cinnamon, and deeper parts of the medium slightly golden brown.

*Stromata and sclerotia.*¹ Plectenchymatic upgrowths of variable occurrence, commonly 1-4, but sometimes none, in single-conidium cultures; they appear as rugose discs on the plectenchymatic surface, 2-3 mm. across, brown, sometimes bluish green; at 2 months they may be 2 mm. high, with rugose or tuberculate surface, and of chocolate brown or greenish blue colour superficially.

Sporodochia. Scattered patches on the more open mycelium to clustered patches on denser mycelium; increase to masses 5 mm. in size or spread to pseudopionnotal patches; in colour light salmon or apricot buff at first to pinkish cinnamon at 3-4 weeks. Also on sclerotial bases in the plectenchymatic layer or on the tubercles of stromata; these sporodochia commonly of brownish shades by diffusion of colour from the bases.

Conidia. (a) Microconidia in aerial mycelium mainly of macroconidial shape, but with blunt apex and less curvature; mainly 3-septate; 0-, 1-, 2-septate sometimes 10 per cent., and 4- and 5-septate sometimes 30 per cent. Pyriform conidia have not been observed, except occasional large ones on maize-meal agar.

(b) Macroconidia are curved, typically broader at one-third of the length from the apex, apex slightly bulbous, pedicel well developed. Some closely resemble conidia of *F. culmorum*, especially those on incubated infected material.

(1) Sporodochial, culture 3 weeks old. 3-septate, 10 per cent.; average 30.1×4.7 ($26.0-36.5 \times 3.5-5.2$) μ . 4-septate, 15 per cent.; average 32.3×4.8 ($28.6-36.5 \times 4.0-5.2$) μ . 5-septate, 75 per cent.; average 41.5×5.1 ($33.8-49.4 \times 4.6-5.5$) μ . 6-septate, occasional.

(2) Sporodochial, culture 3 months old. 3- and 4-septate, occasional. 5-septate, 98 per cent.; average 42.9×5.3 ($33.8-52.0 \times 4.8-6.0$) μ . 6-septate, 1 per cent.; average 48.1×5.4 ($41.6-52.0 \times 5.2-6.0$) μ .

(3) Sporodochial, from wheat bases incubated 10 days. 3-septate, 75 per cent.; average 31.0×5.07 ($28.5-34.0 \times 5.0-5.2$) μ . 4-septate, 20 per cent.; average as 3-septate. 5-septate, 5 per cent.; average 33.3×5.3 ($31.0-37.5 \times 5.2-5.5$) μ .

(4) Sporodochial, from wheat bases incubated 4 weeks. 3-septate, 10 per cent. 4-septate, 20 per cent. 5-septate, 70 per cent. One or both of the terminal cells of

¹ There is no line of demarcation between these structures as the terms are used with reference to *Fusarium*. Plectenchymatic upgrowths may remain small, with more brittle external layers and softer inner pseudoparenchymata; these are sclerotia, and may, or may not, become blue externally. When similar upgrowths continue to increase in size they may, or may not, become more or less stipitate, and they usually have a tuberculate surface; these are stromata. The tubercles of these stromata are equivalent to the smaller growths called sclerotia, and may, or may not, become blue on the exterior side. Sclerotia and stromatic tubercles, whether blue or not, may bear spores or sporodochia, or remain sterile.

most conidia empty or disappeared, and the remaining cells of pseudo-chlamydospore form.

(5) Sporodochial, from seven kinds of substrata. 3-septate, average $28.2 \times 4.6 \mu$. 4-septate, average $30.9 \times 4.9 \mu$. 5-septate, average $37.1 \times 5.1 \mu$. 6-septate, average $47.4 \times 5.3 \mu$ —rare.

Perithecial structures. These are clearly defined in cultures about 3 months old, and appear as bluish dots on the tubercles of stromata and around the edges of sclerotial discs, singly or in small clusters, globose or with apical protuberance blue-violet whilst the basal part is still brownish; $70-80 \times 90-100 \mu$, devoid of contents (see below).

Chlamydospores. Rare; found only in matted mycelium of cultures on maize-meal agar, and to small extent on sterile straw, after 3 months; in short chains, terminal and intercalary, smooth, faintly tinted cinnamon, of average diameter 9.5μ . No conidial chlamydospores have been observed, but pseudochlamydospore segments are common in old conidia.

Other media. Hard oat agar favours more abundant aerial mycelium, and sporodochia with 3-septate conidia up to 95 per cent.; average 29.6×4.2 ($23.5-36.5 \times 3.3-5.2$) μ . Cooked potato bears abundant aerial mycelium, white with shades of sulphine yellow to pale olive lake; stromata are numerous and large (up to 6 mm. or more in diameter) after 3 months, cinnamon to mummy brown in colour, and rarely bearing perithecial structures; no odour when these cultures are exposed to air (see *forma* 1).

The Gibberella stage. In *F. Sambucinum* the blue aggregates are of perithecial nature. Their occurrence in cultures on wheat-meal agar is mentioned above; here, as on other artificial media, they occur on the tubercles of stromata, but are more common around the edges of disc-like, undeveloped stromata (sclerotia) which are usually surmounted by sporodochia also. On sterile wheat straw they occur singly, or in small clusters, in the sparse, superficial mycelium, apart from any plectenchymatic structures; they are at first brownish with obvious cellular outer wall, and later blue-violet, nearly spherical $78 \times 78 \mu$, or egg-shaped $68-73 \times 96-104 \mu$. On cooked wheat grains in moist, lined Petri dishes, the structures are abundant on the moist paper, rather longer, and of perfect perithecial form, $195 \times 225-200 \times 240 \mu$. Every attempt to bring these perithecia to spore-bearing condition, including growth on natural materials in tubes and dishes of glass transmitting ultra-violet rays, has failed; they remained brittle, and either devoid of contents or with granular matter expelled under pressure. It is recorded in *Fusarium Monographie* (7d, p. 355) that *Gibberella pulicaris* (Fr.) Sacc. has yielded the conidial form *F. Sambucinum*, but there is no record that the conidial stage has produced the perithecial form, mature or immature. It would appear that the British strain of *F. Sambucinum* has considerable latent capacity for the production of the perfect stage.

Chief cultural characters. Microconidia of aerial mycelium not abundant, mainly 3-septate with minutely bulbous apex; none pyriform. Stromata brownish, 2-3 mm. in diameter and height, with rugose or tuberculate surface; some tubercles tipped with blue perithecial structures, some with sporodochia, others sterile. Perithecial structures also associated with sclerotial discs on the plectenchymatic layer. Macroconidia dominantly either 3- or 5-septate according to conditions, and of characteristic shape. Chlamydospores rare, and in old cultures only.

Pathogenicity. Not yet investigated, but the conditions under which this species has been found repeatedly do not suggest any marked parasitic capacity.

F. Sambucinum Fuckel *forma* 1 Wr.

The best known of the several names under which this fungus (Text-fig. 6) has been recorded is *F. polymorphum* Matruchot. A brief diagnosis, establishing the new nomenclature, is given in *Fusarium Monographie* (7d), p. 356). The organism has been recorded from decaying grasses, trees, fruits, and from living Brassicæ, hop, some shrubs, rye and maize, and soil humus. The present writer isolated the fungus repeatedly from the bases and culms of wheat, and basal parts of oats and perennial rye grass of leys, the form being consistently as here described. Infected material when incubated yields definite mycelial growth bearing microconidia of very varied forms: 0-, 1-, and 2-septate pyriform, and occasionally 3-septate sickle-shaped ones; these microconidia closely resemble those of *F. Poae* Peck. Later, small sporodochia occur on the substrata, the conidia of which show considerable resemblance to those of *F. culmorum*.

Characteristics of single-conidium cultures on wheat-meal agar.

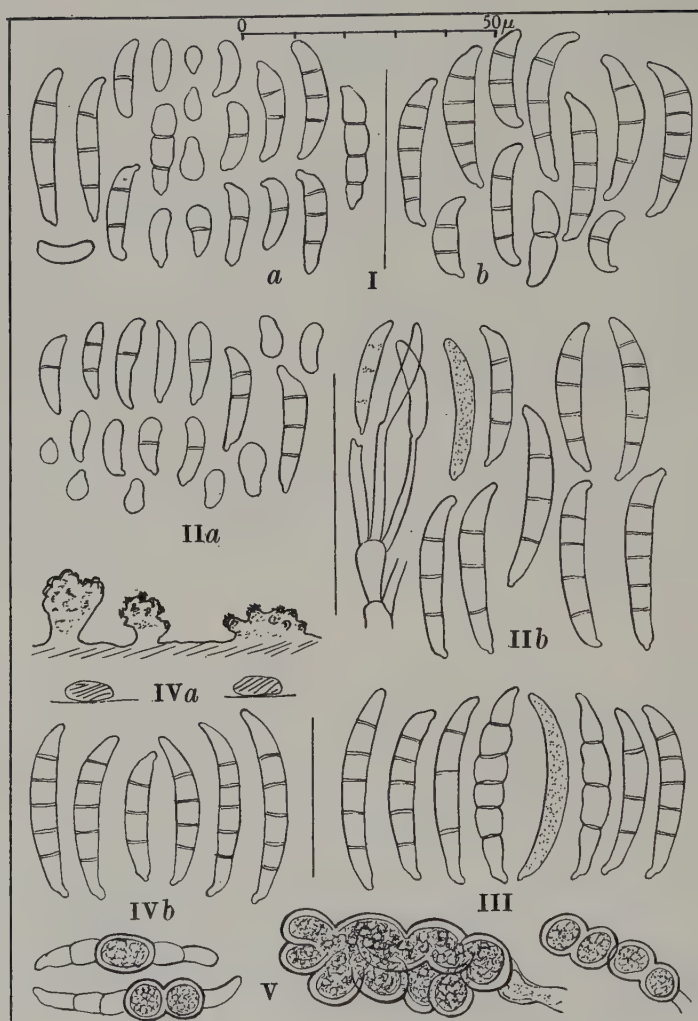
Aerial mycelium. Well developed but not abundant, white, floccose but soon matting and then showing the beginnings of sclerotial discs bearing sporodochia forming in exuded water droplets.

Substratum. No decided coloration; eventually about honey yellow throughout. In subcultures small areas of Etruscan red may arise.

Stromata and sclerotia. Plectenchymatic humps, tough but not brittle, up to 3 mm. in diameter, with tuberculate surface, golden brown in colour; the tubercles commonly bear exuded water droplets and the tips become blue; in some of the droplets sporodochia are deposited and mask the blue tips. Smaller plectenchymatic structures, pimple-like, about 1 mm. across, occur on the plectenchymatic layer and on the matted mycelium; they correspond to the tubercles of stromata, and like them may become blue or remain honey yellow, and may bear sporodochia deposited in water droplets or remain sterile (see notes).

Sporodochia. Most common on and around stromata, there forming small pseudopionnotal patches, of salmon orange to apricot buff colour; also singly or in tuberculate clusters on the stromatic tubercles and sclerotia mentioned above, and in mycelium, 1-3 mm. in diameter and height, and of brownish or bluish green shades according to diffusion of colour from the base. Occasionally sporodochia envelop sclerotia, the structures globular to reniform, smooth and shiny, and dark green in colour, having the appearance of large sclerotia.

Conidia. (a) Microconidia: the numerous pyriform conidia amongst others in aerial mycelium, especially about the bases of stromata, are notable; of the sickle-shaped conidia 1-septate average $19.6 \times 5.5 \mu$, and 3-septate $22.4 \times 5.0 \mu$.



Text-fig. 6. *Fusarium Sambucinum* Fuck. forma 1 Wr.

- I. From natural material incubated; (a) microconidia from aerial mycelium; (b) forms occurring in sporodochia.
- II. From wheat-meal agar; (a) microconidia of aerial mycelium at 1 month; (b) macroconidia of sporodochia at 2 months.
- III. From wheat-meal agar; pseudopionnotal, culture 2 months old.
- IV. From hard oat agar; (a) stromata, and sclerotia enveloped by sporodochial layer (see text and compare *F. tricinctum*, Text-fig. 8); (b) sporodochial, macroconidia from culture 6 weeks old. [(a) not to scale.]
- V. Chlamydospores, conidial and mycelial, from subcultures on hard oat agar.

(b) Macroconidia not strongly curved, widest at one-third the distance from the apex or of nearly uniform width throughout, apex generally slightly bulbous, and pedicel distinct.

(1) Sporodochial, culture 6 weeks old. 3-septate, 12 per cent.; average 29.4×4.5 ($23.8-39.8 \times 4.0-5.3$) μ . 4-septate, 5 per cent.; average 35.5×4.9 ($30.0-40.5 \times 4.5-5.3$) μ . 5-septate, 83 per cent.; average 38.1×4.9 ($37.5-45.0 \times 4.7-5.0$) μ .

(2) Sporodochial, blue from sclerotial base, culture 10 weeks old. 3-septate, 70 per cent.; average 32.6×4.7 ($26.5-39.8 \times 3.9-5.3$) μ . 4-septate, 20 per cent.; average 36.1×5.0 ($31.8-39.8 \times 4.9-5.3$) μ . 5-septate, 10 per cent.; average 35.5×5.0 ($31.8-39.8 \times 4.0-5.3$) μ .

(3) Sporodochial, from mycelium, culture 3 months old. 3-septate, 2 per cent.; average 28.9×4.5 ($27.5-32.5 \times 4.4-4.9$) μ . 4-septate, 5 per cent.; average 35.5×4.85 ($32.5-40.0 \times 4.8-5.0$) μ . 5-septate, 90 per cent.; average 37.8×4.9 ($32.5-42.5 \times 4.7-5.0$) μ . 6- and 7-septate, occasional; average 41.5×4.9 ($39.0-47.5 \times 4.9-5.1$) μ . 8-septate, rare, one measured, 40.0×5.0 μ .

(4) Sporodochial, from seven kinds of substrata. 3-septate, 33 per cent.; average 30.7×4.75 μ . 4-septate, 13 per cent.; average 34.2×4.95 μ . 5-septate, 52 per cent.; average 36.8×4.93 μ . 6-septate, 1 per cent.; average 41.1×4.95 μ . 7- and 8-septate occasional, sizes as 5- and 6-septate. There is a distinct change in septation mode with age, but little difference in average size.

Chlamydospores. Rare in single-conidium cultures, but fairly numerous in mycelial growth of subcultures especially on hard oat agar; mainly terminal, but also intercalary, singly, in chains, and in small clusters; commonly $7.6-12.5$ μ in diameter, but some up to 15×10 μ . Rare in conidia, and intercalary ones only observed, $7 \times 7-8 \times 11$ μ diameter; cinnamon tint to cinnamon colour.

Other media. Hard oat agar inoculated with mycelial material bears large stromata, 5 mm. or more high and approaching stipitate form; the dark green, reniform sporodochia are more frequent on this medium, and mycelial chlamydospores are numerous in cultures 3 months old. Pseudopionnotal patches are often mineral red, vinaceous lilac, or vinaceous purple in cultures 1 month old. Cooked potato cultures about 6 weeks old have a decided odour like bruised hemlock when exposed to the air; inoculation with sclerotia-bearing microconidia causes the surface to be of duck green colour like an expansive sclerotium. Sterile wheat straw produces remarkably uniform macroconidia.

Notes. The sclerotia in this species form show no resemblance to perithecia, hence it may be assumed that these simple aggregates are a degenerate form of the perithecial structures described under *F. Sambucinum*. The ascigerous stage of *F. Sambucinum* f. 1 is given as *Gibberella pulicaris* (Fr.) Sacc. var. *minor* Wr. (*Id.*, p. 356), but the writer is not aware of any cultural investigations in which the conidial and perithecial forms have been evolved one from the other.

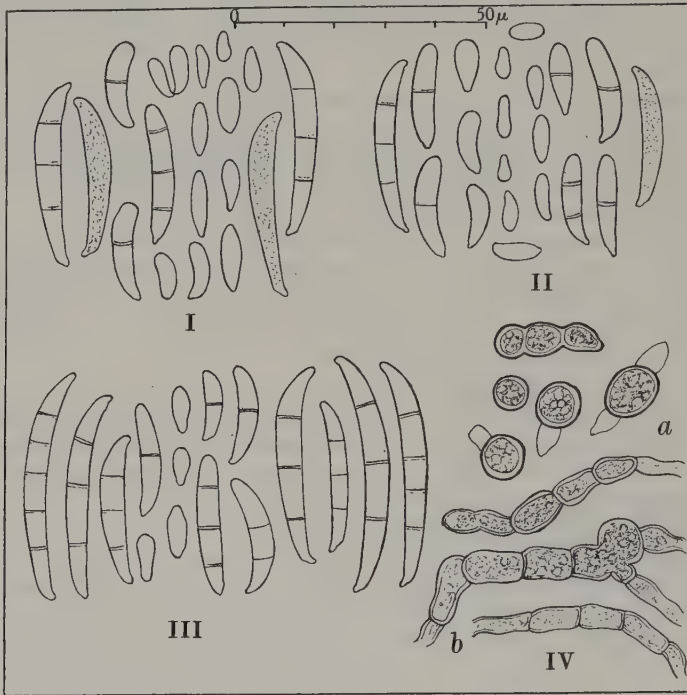
Chief cultural characters. Microconidia abundant in aerial mycelium, pyriform ones being obvious. Stromata are well developed on most media, sometimes stipitate. Sclerotia honey yellow, or blue-green to blue-violet, the latter showing no resemblance to perithecia. Macroconidia are dominantly 5-septate, and of characteristic "discolor" shape. Chlamydospores present.

Pathogenicity. Trials during one season only with wheat, barley and oats grown from clean seed in contaminated soil, and from contaminated

seed in clean soil, indicate that *F. Sambucinum* f. 1 is of little, if any, importance as a foot rot organism in cereal cultivation.

F. trichothecioides Wr.

This fungus (Text-fig. 7) was described by Wollenweber (^(7b), p. 206) from its occurrence as a wound parasite on rotting tubers of *Solanum*



Text-fig. 7. *Fusarium trichothecioides* Wr.

- I. From wheat-meal agar; conidia in the compact mycelial layer, culture 3 weeks old; 98-99 per cent. of "blunt" type, 1-2 per cent. "discolor" type.
- II. From wheat-meal agar; culture 3 weeks old; from aerial mycelium at top of slant, "discolor" type rare.
- III. From wheat-meal agar; sporodochial, culture 2 months old; "discolor" type about 80 per cent.
- IV. (a) Chlamydospores in conidia, culture 3 months old; (b) hyphal segments often resembling chlamydospores.

tuberosum, and it has been recorded by Sherbakoff (⁽⁶⁾, p. 229) from similar sources. The present writer has found it several times on basal parts of wheat and barley grown in the eastern and southern parts of England. On incubated infected material the fungus appears as a very sparse mycelium bearing numerous microconidia, and once a few minute,

cream-like sporodochia appeared on material so kept for two months, these consisting mainly of macroconidia. A difference in first cultures will occur according to whether micro- or macroconidia are used, as stated below. As natural material is generally overrun by other organisms before sporodochia appear, single conidium cultures will generally be prepared from microconidia, and such cultures are described here.

Characteristics of single-conidium cultures on wheat-meal agar.

Aerial mycelium. Scanty on the lower part, a thin loose layer on the upper part, to short floccose growth at the top of slants; white. Later, white tufts or masses develop in places, especially toward the base.

Substratum. No definite coloration; a very slow change proceeds uniformly through the medium to faint pinkish cinnamon then light ochraceous tawny colours.

Sporodochia. Appear in from 4 to 6 weeks, pimple-like, 1 mm. in size, scattered on the surface of the medium and visible through the thin mycelial layer; cream, later salmon buff in colour; each sporodochium is on a base of compact, interwoven hyphae.

Conidia. (a) Microconidia; in the floccose aerial mycelium 0- and 1-septate, from ovate to elongate-reniform; in mycelium on the surface of the medium two types:

(1) "blunt" type, 98-99 per cent. 0-septate, 98 per cent.; average 10×3.5 ($7.5-15 \times 3.0-3.9$) μ . 1-septate, 2 per cent.; average 16.5×4.0 ($15.0-27.5 \times 3.5-4.5$) μ . 2-septate, occasional. 3-septate, occasional; average 25.0×4.5 ($21.0-29.5 \times 3.5-5.0$) μ .

(2) "discolor" type, 1-2 per cent. 0- to 3-septate indistinctly; average 39.0×4.97 ($28.6-46.8 \times 3.9-5.4$) μ .

(b) Macroconidia of sporodochia also of two types.

Sporodochial, culture 2 months old.

(1) "blunt" type about 20 per cent.; of these: 0-septate, ovate to oblong, 60 per cent.; ovate, $5.0-7.0 \times 3.0-3.3$ μ . Oblong, average 11.7×4.0 ($10.0-12.5 \times 3.5-4.9$) μ . 1-septate, usually slightly curved, 35 per cent.; average 20.3×4.2 ($13.0-26.0 \times 3.9-4.7$) μ . 2-septate, very few. 3-septate, slightly curved, broadly rounded apex, papillate base; average 27.5×4.7 ($26-34 \times 4-5$) μ .

(2) "discolor" type about 80 per cent.; sickle-shaped, usually slightly constricted at the apex, and pedicellate or papillate at the base. 3-septate, 98 per cent.; average 43.0×4.66 ($33.5-54.5 \times 3.9-5.2$) μ . 4-septate, 1 per cent.; average 51.6×5.0 ($46.8-54.5 \times 4.9-5.1$) μ . 5-septate, 1 per cent.; average 53.5×4.7 ($52.0-55.0 \times 4.0-5.0$) μ .

Chlamydospores. In conidia amongst aerial mycelium of cultures 2 or more months old; usually single and intercalary, but occasionally 2 or 3 together; rare; $6 \times 6-10 \times 11$ μ ; not coloured. Coarse, granular hyphae with enlarged segments are common, these segments sometimes resembling chlamydospores.

Notes. The description given is for the growths most frequently arising from single conidia from natural sources; they may be considered intermediate types. The extreme types are perfectly well differentiated in single-conidium subcultures from artificial sources, the following features appearing on agar media and cooked potato.

(a) A mycelial type: aerial mycelium well developed over the whole substratum, white, the surface of the substratum cream or ivory coloured; conidia abundant, almost

entirely 0- and 1-septate ovate, oblong, or slightly curved "blunt" type; occasional conidia of "discolor" type present.

(b) A reduced mycelial type, the aerial mycelium thin and sparse scarcely masking the substratum; the surface of the substratum slightly cinnamon tinted; conidia abundant, almost entirely "discolor" type with granular contents and indistinct septation; occasional conidia of "blunt" type present.

These types must be noted because either or both of them can, and do, appear to greater or lesser extent in first cultures from natural sources.

Chief cultural characters. Aerial mycelium white, thin on the lower parts of slant cultures, somewhat woolly to floccose on the upper parts. Conidia, distributed throughout the mycelium, of two types, blunt and discolor. Some first cultures are more highly developed in the mycelial and microconidial direction, others in the pseudopionnotal and macroconidial direction. Chlamydospores rare, and in conidia of old cultures only.

Pathogenicity. Not investigated.

SECTION SPOROTRICHIOIDES.

F. tricinctum (Cda.) Sacc.

The synonyms of this fungus (Text-fig. 8) given in *Fusarium Monographie* (7d, pp. 437, 459) are: *F. heterosporum* Ellis; *F. helianthi* Lewis, *F. roseum* (pr.p.) Link. It has been recorded under one or other of the above names from barley, rye, maize, apple, and thistle. The present writer has found it of very common occurrence, apparently mainly or entirely saprophytic, on the aerial and underground parts of wheat, barley and oats, as well as on rye grasses, turf grasses, pea, bean, viola, and strawberry. When infected material is incubated considerable aerial mycelium is produced bearing microconidia of various forms: 0- and 1-septate pyriform, sporotrichial, and allantoid; and 1- to 3-septate sickle-shaped. Later these microconidia become rare, and small sporodochia with 3-septate macroconidia develop.

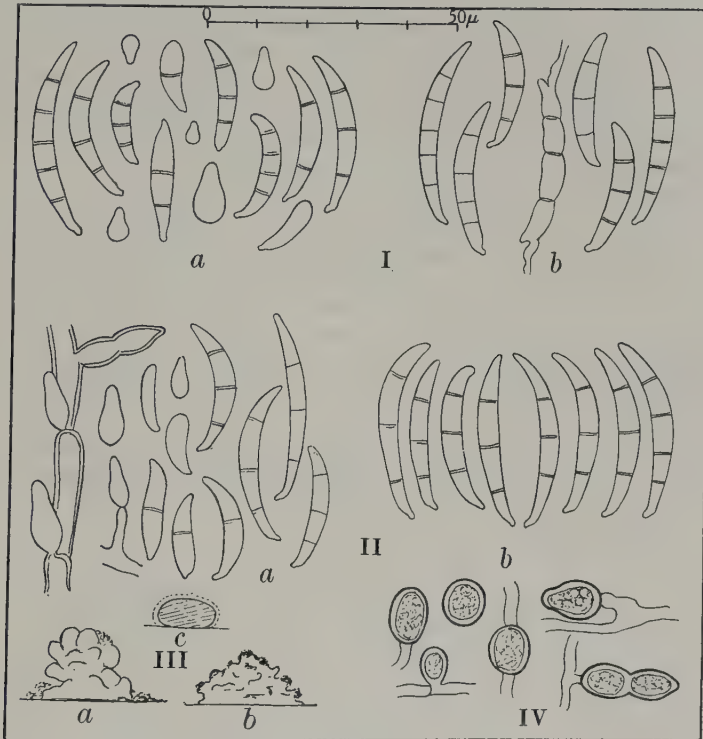
Characteristics of single-conidium cultures on wheat-meal agar.

Aerial mycelium. Well developed, woolly, white with ochraceous and pomegranate purple tints in parts; the layer next the substratum tinted ochraceous with carmine.

Substratum. Plectenchymatic layer amaranth to pomegranate purple, and later Bordeaux purple; the deeper layers gradually become brownish yellow, and after about 3 months pale sayal brown.

Stromata and sclerotia. Nodules erumpent from the plectenchymatic layer, tough and somewhat brittle, honey yellow; tuberculate, the tubercles either blue tipped, bearing sporodochia, or sterile; 3-5 mm. diameter at 6 weeks, and 10 mm. in diameter and 5 mm. high at 3 months. Stromata do not appear in all cultures, but when present number from 1 to 4 per slant. Smaller structures, sclerotia, 0.5-1 mm., occur in the mycelium; they may remain of honey yellow colour, or become blue-green in the outer layers.

Sporodochia. Apricot buff to apricot orange in colour, 0·5–1 mm. in size; on the tubercles of stromata, on small sclerotia, and on mycelium without sclerotial bases especially on the sides of stromata. Sometimes a sporodochial layer on tubercle or sclerotium becomes blue-green by diffusion of colour from the latter.



Text-fig. 8. *Fusarium tricinctum* (Cda.) Sacc.

- I. From bases of wheat plants incubated; conidia (a) miscellaneous, (b) typical.
- II. From wheat-meal agar; cultures 6 weeks old; (a) microconidia of aerial mycelium; (b) macroconidia of sporodochia.
- III. From hard oat agar; stromata (a) sterile tubercles with associated sporodochia, (b) blue tipped; (c) sclerotium enveloped by sporodochial layer (compare *F. Sambucinum* forma 1, Text-fig. 6); not to scale.
- IV. Chlamydospores; from cultures up to 6 months old.

Conidia. (a) Microconidia on aerial mycelium mainly 0- to 1-septate pyriform, lemon-shaped, and allantoid mainly.

(b) Macroconidia in sporodochia 3-septate up to 99 per cent. in young cultures, with increase of 4- and 5-septate as these become drier; of typical sickle shape with well marked curvature.

(1) Sporodochial, culture 6 weeks old. 3-septate, 99 per cent. including those indistinctly septate; average $33\cdot1 \times 3\cdot96$ ($27\cdot5-40\cdot0 \times 3\cdot5-4\cdot2$) μ .

(2) Sporodochial, culture 3 months old. 3-septate, 97 per cent.; average 30.9×3.7 ($26-39 \times 2.7-4.5$) μ . 4-septate, 2 per cent.; average 35.4×4.3 ($31.2-39 \times 3.9-4.6$) μ . 5-septate, occasional.

(3) Sporodochial, from blue sclerotial base, culture 3 months old. 3-septate, 80 per cent.; average 30.4×3.75 ($23.5-34 \times 2.7-4.3$) μ . 4-septate, 15 per cent.; average 27.8×4.2 ($26-34 \times 4.0-4.5$) μ . 5-septate, 5 per cent.; average 33.8×3.9 ($26-36 \times 3.5-4.5$) μ .

(4) Sporodochial, from five kinds of substrata, cultures 6 weeks to 4 months old. 3-septate, average 33.7×4.0 ($22.5-42.5 \times 2.6-4.9$) μ . 5-septate, 0-5 per cent., average 1 per cent.; in exceptional cases, as when wheat bases were kept in moist chambers for 4 months, 5-septate increase to 30 per cent., and may attain an average size of 38×4.4 μ ; the increase in septation mode with age is clear.

Chlamydospores. Rare, and found only in the aerial mycelium of cultures 2-4 months old derived from mycelial inoculum; $7.5-15$ μ in diameter, smooth walled, single or in short chains, usually terminal but occasionally intercalated in hyphae.

Other media. Cooked potato frequently yields aerial mycelium in which there are patches of Prussian green tint; pyriform conidia are usually abundant, and sclerotia, 1-2 mm. in diameter are greenish blue to blue-black. Sterile wheat straw bears small sporodochia of very uniform conidia, at 6 weeks as follows: 3-septate, 85 per cent.; average 35.5×3.9 ($27-43 \times 3.6-4.2$) μ . 4-septate, 15 per cent., and 5-septate, occasional, average 36.4×4.3 μ .

Notes. Chlamydospores are rarely to be found in any but first cultures; pyriform microconidia also disappear rapidly in subcultures. No pseudopionnotal form is obtained in subcultures derived from sporodochial inoculum, nor a more distinctly mycelial type from mycelial inoculum.

Chief cultural characters. Aerial mycelium bearing, amongst others, pyriform 0- (some 1-) septate microconidia. Macroconidia 3-septate, curved, average 33.7×4.0 μ . Stromata large, frequently blue tipped, and sclerotia, 1-2 mm., blue-green. Chlamydospores in hyphae of old cultures only.

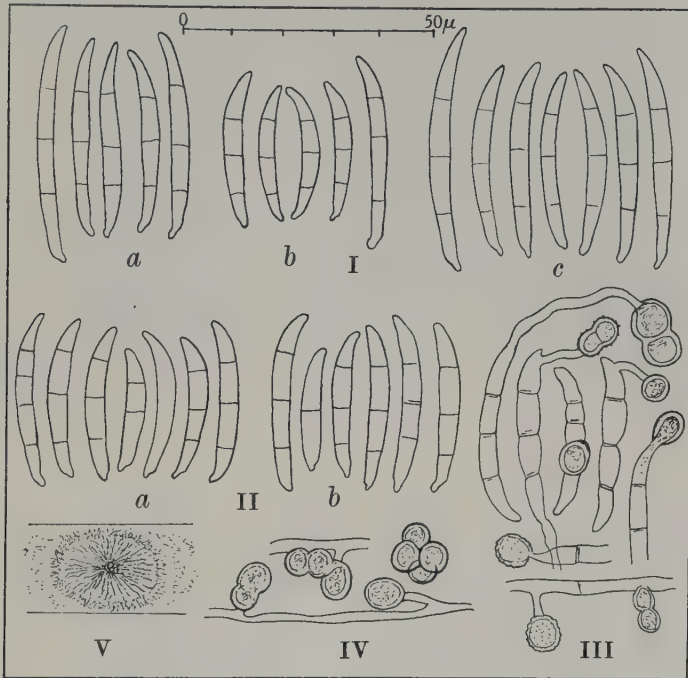
Pathogenicity. Experiments with wheat, barley and oats up to seedling stages did not show *F. tricinctum* to be pathogenic; nor has this species been found naturally under conditions indicating more than saprophytic or weakly parasitic capacity. It cannot be considered a primary cause of failure of cereal plants.

SECTION EUPIONNOTES.

F. merismoides Cda. var. *majus* Wr.

The many synonyms of this fungus (Text-fig. 9) are given in *Fusarium Monographie* (7d), p. 307), the best known, because used in the more recent literature being *F. udum* (Berk.) Wr. Butler (3b) used the name for the causal organism of the pigeon-pea wilt, but Wollenweber considers this a different species (7b), p. 38). Under the name of *F. udum* the fungus has been recorded from the cut surfaces and bark of oak, elm and other trees; from roots, tubers and bulbs of beet, Brassicæ, dahlia, potato, tulip; from the culm of maize and seed of wheat and rye. The

present writer has found it repeatedly on the basal parts of wheat and oats, grain of barley, and in turf from lawns etc. When infected material is incubated there is generally a mere trace only of superficial hyphae, but numerous small sporodochia (0.5 mm.) or small patches (2 mm.) of cream colour, consisting of 3-septate conidia.



Text-fig. 9. *Fusarium merismoides* Cda. var. *majus* Wr.

- I. From parts of plants incubated; (a) wheat bases, (b) barley ear, (c) cabbage roots.
- II. From wheat-meal agar; (a) sporodochial, culture 2 months old; (b) pseudopionnotal, culture 1 month old.
- III. Chlamydospores produced in water.
- IV. Chlamydospores in mycelium of culture on wheat straw, culture 18 months old.
- V. Macroscopic appearance of single-conidium culture; not to scale.

Characteristics of single-conidium cultures on wheat-meal agar.

Aerial mycelium. None; but very delicate hyphae, visible under a microscope, occur superficially on the submerged growth mentioned below.

Pionnotes. A circular growth around the point of inoculation, with zonation and radiate striae more or less clear, forming a pionnotal area, of pinkish buff, light pinkish cinnamon, light salmon orange or bittersweet pink colour according to the temperature and the intensity of the light to which exposed. The pionnotes is bordered by a narrow margin of radiate, submerged, hyaline hyphae; as the cultures age the

submerged growth continues to extend, forming a tough, skin-like layer without superficial pionnotes.

Sporodochia. These arise on the submerged growth of older cultures mentioned above; singly 0.5 mm., or in small clusters or patches (3 mm.), of creamy buff to ochraceous salmon colour. The conidia are much more uniform and more clearly septate than those in the pionnotes.

Substratum. No decided coloration; the medium from light to dark chamois shade at about 2 months.

Conidia. Typically 3-, occasionally 4-, very rarely 5-septate; 6- and 7-septate conidia occur on salts glycerine agar but are exceptional.

(1) Pionnotal, culture 1 month old. 3-septate, up to 100 per cent.; average 37.5×3.9 ($27.5-42.5 \times 3.5-4.4$) μ .

(2) Sporodochial, culture 2 months old. 3-septate, up to 95 per cent.; average 30.7×3.7 ($26-36 \times 3.5-3.9$) μ .

(3) Sporodochial, on wheat bases incubated 3 weeks. 3-septate, up to 99 per cent.; average 40.5×3.1 ($36-41 \times 2.7-4.0$) μ .

(4) Pionnotal and sporodochial from eleven substrata. 3-septate, 85 per cent.; average 35.7×3.55 μ . 4-septate, 5 per cent. approximately. 5-septate, 0.1 per cent.; average 45.0×4.3 μ .

Chlamydospores. None on wheat-meal agar, but in small number in conidia of old cultures on sterile wheat straw, and occasionally in conidia and hyphae on salts glycerine agar; terminal and intercalary, singly or in chains or clusters of 2-4. They develop very freely within 48 hours from conidia suspended in water, especially when the conidia are from natural sources or from substrata of low nutrient value, *e.g.* straw, hard potato agar. These chlamydospores are generally borne on short outgrowths and some have a finely echinulate epispor. The size varies according to the source of production; 5×6 μ from old cultures, $6 \times 8-8 \times 9$ μ in or from conidia in water, and up to 8×10 μ on natural substrata.

Other media. Salts glycerine agar, pH 6.6, bears a pionnotes of colour varying from orange pink at the periphery to buff at the centre, and zonation by confluent sporodochia deposited concentrically on the radiate striae is clearly shown. Hard potato agar with 5 per cent. of dextrose gives minute sporodochia singly but closely on evanescent, radiate hyphae; a complete pionnotes is not formed, since these sporodochia remain scarcely or not at all confluent. This medium also, more frequently than others, bears erect, coremia- or bristle-like growths, of ivory to creamy white colour; they arise from the centre of the pionnotal area.

Notes. The variety *majus* is characterised by rather longer and narrower conidia than the type species *F. merismoides*, and by the occurrence of 4-7 septa. This higher septation mode is not common in British isolants; incubated wheat bases have yielded 4-septate up to 5 per cent. (average 41.6×3.25 μ), 5-septate occasionally (average 46.8×3.5 μ), and very rarely 6- and 7-septate. Some media, *e.g.* hard potato agar and salts glycerine agar, also bear 6- and 7-septate conidia, but they are always abnormal forms.

Chief cultural characters. The 3-septate macroconidia which readily produce chlamydospores in water, and yield a characteristic pionnotes in culture aid easy recognition.

Pathogenicity. Not yet investigated.



Fig. 1.

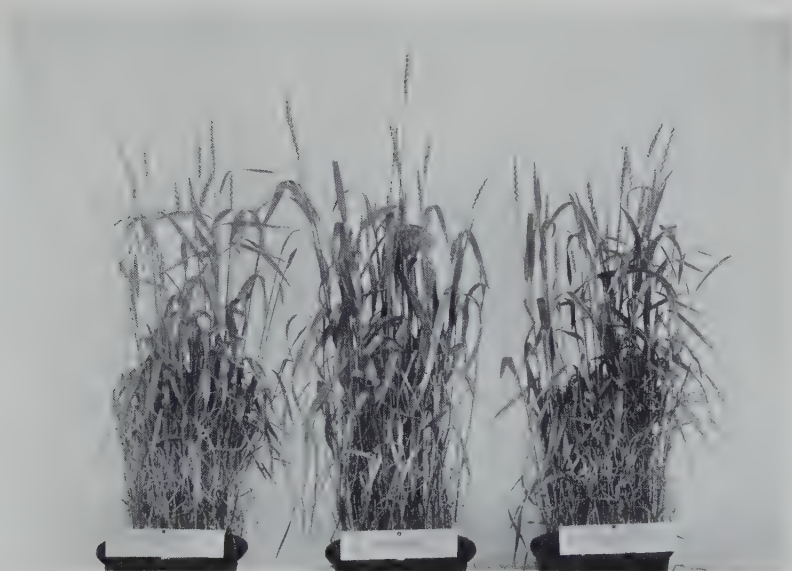


Fig. 2.

BENNETT.—FUSARIUM SPECIES ON BRITISH CEREALS (pp. 479-507).



Fig. 1.



Fig. 2.

BENNETT.—FUSARIUM SPECIES ON BRITISH CEREALS (pp. 479-507).

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EXPLANATION OF PLATES XXI AND XXII.

PLATE XXI.

Fusarium Equiseti (Cda.) Sacc.

(The name on the labels was then (1930) the accepted nomenclature.)

Fig. 1. Inoculation of ears, remaining in moist atmosphere from 3 to 7 days, reduced the germination capacity by from 25 to 75 per cent. The grains remaining viable yielded poorer and thinner seedlings owing to foot rot, as shown.

Fig. 2. Wheat grown in contaminated soil or from contaminated seed yields a poor crop (see text).

PLATE XXII.

Fusarium Equiseti (Cda.) Sacc. forma 1 Wr.

(The name on the labels was then (1930) the accepted nomenclature.)

Fig. 1. Inoculation of ears in different stages of maturity proved susceptibility at all stages. Viable grains from such ears gave poorer and thinner seedlings as shown, most of them affected with foot rot.

Fig. 2. Wheat grown in contaminated soil or from contaminated seed, although affected with foot rot, showed scarcely marked difference during growth; but plants "ripened" rather earlier and size and weight of grain were reduced.

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FURTHER STUDIES ON QUANTITATIVE METHODS WITH TWO PLANT VIRUSES

BY GEOFFREY SAMUEL

*(Rothamsted Experimental Station, formerly Waite
Agricultural Research Institute),*

R. J. BEST

*(Waite Agricultural Research Institute,
University of Adelaide)*

AND J. G. BALD

*(Division of Plant Industry, Council for Scientific
and Industrial Research, Australia).*

(With Plate XXIII and 1 Text-figure.)

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I. INTRODUCTION.

DURING the course of work on the inactivation *in vitro* of the virus of tomato spotted wilt, it became necessary to examine in greater detail some of the factors influencing the estimation of virus concentration by the primary lesion method (5, 6, 8). On the one hand were statistical problems concerned with the arrangement of experiments and the reduction of data, and on the other hand were factors of unknown magnitude

such as the physico-chemical effects of electrolytes added to the inoculum, differences of pH , temperature, etc., all of which might affect the numbers of lesions and the estimates of virus concentration. These problems were examined only so far as was necessary for standardising the technique and making reliable the interpretation of results in the work on inactivation. The data obtained, which are summarised in the present paper, suggest that more detailed studies on some of the points which have arisen would be profitable. Most of the work reported here was carried out in 1933.

In much of the earlier work done with plant viruses the crude expressed sap from infected plants was employed, usually diluted in water to varying extent, but with no determinations of hydrogen-ion concentration or other physico-chemical properties. Of recent years, since more quantitative work has been undertaken, improvements have been made both in the direction of purifying viruses and of determining the hydrogen-ion concentration of the samples employed. In some of the most recent work dilution has been carried out with physiological saline, Ringer's solution, etc. (3), or with a phosphate buffer adjusted to pH 7 (7). The adjustment and control of hydrogen-ion concentration involves the addition of buffer salts to the inoculum, but no studies appear to have been made as yet on the effect of electrolyte content on the numbers of primary lesions produced. This question was first studied with tobacco mosaic and the results for this virus are given here; later experiments of a similar kind have been done with tomato spotted wilt.

II. EXPERIMENTAL TECHNIQUE.

(1) *The arrangement of experiments.*

As a result of the need which developed for comparing as many as a dozen virus samples at a time replication in randomised blocks and Latin squares was adopted. Also since much of the work had to be done with virus samples which were changing in concentration at an appreciable rate during inoculation it became a problem as to how far the half-leaf method could advantageously be combined with the randomised arrangements. Finally the whole question of arrangement was examined by analysis of a uniformity trial that had been made with the virus of tobacco mosaic on *Nicotiana glutinosa*. Since Youden and Beale have recently published an account of similar work (8), and as their results agree closely with our own, there is no need to duplicate their data. The results

common to both will be briefly summarised and certain additional points mentioned.

Youden and Beale found that there was a gradient of susceptibility to infection between different leaf positions on a plant. The nature of the gradient varied with different sets of plants, but within any group of plants raised under the same conditions there was a strong correlation of lesion count with leaf position. The gradient was not so noticeable if the physiological characters of the leaves were rendered more uniform by removing the growing tip some days before inoculation, a procedure first advocated by Holmes(5). We have also noticed that greater uniformity appears naturally in leaves of plants growing under reduced light intensity.

In estimating the significance of the results obtained by the primary lesion method the variance attributable to experimental error may be considerably reduced by calculating and removing the variance due to differences between plants and between leaves of different ages. The possibility of eliminating these sources of variation depends on the experimental arrangement. If more than two treatments are to be compared Youden and Beale advocate a rotated arrangement, using a leaf as the experimental unit, the several treatments being so distributed among the leaves that each appears equally often on each plant and at each leaf position. The present writers also arranged the replicates so that whenever possible the variances due to plants and leaf position could be eliminated, but the order was determined by the dealing of cards or by the use of tables of random sampling numbers instead of by rotating the treatments as suggested by Youden and Beale. Complete randomisation is an essential prerequisite for proper use of the analysis of variance as developed by Fisher(4). Also in many cases the number of treatments being compared exceeded the number of suitable leaves per plant, so that all treatments could not be arranged on each plant. This was especially the case in the work with tomato spotted wilt. For this virus the test plants were tobacco, and only two, or at most three, leaves were found to be suitable for inoculation. Usually independent blocks or Latin squares were then planned for leaves of equivalent age, the comparisons often being repeated on leaves of another age.

Since in large experiments, as when sixty or more plants are used, there was always found to be some slight variation in size of plants, the blocks or squares were built up by selecting the largest for the first row, the next largest for the second, and so on, the effect being that columns had practically equal leaf areas. This enabled an examination to be

made for change in strength of inoculum during inoculation, when all inoculations were performed commencing with the plants at one side of the block or square.

Youden and Beale agreed that the half-leaf method is the most accurate for the comparison of two samples of virus, but when more than two samples were to be compared they advocated the use of single-leaf units, arranged in rotation, in preference to any comparison against a standard by half-leaves. The present authors adopted a half-leaf comparison against a standard in a number of their experiments with randomised blocks and Latin squares, and a statistical analysis of the results, which will be presented later, indicates that definite advantages are to be derived from this arrangement in certain cases. In other cases, however, no sufficient improvement was obtained to warrant the extra labour, and single-leaf units were found to be best.

(2) *Choice of plants and method of inoculation.*

The statistical means of eliminating variation were supplemented by care in the raising of young plants, and by the choice of uniform plants raised in one batch for any one experiment. Plate XXIII, fig. 1, shows a desirable standard of uniformity for an 8×8 square of tobacco plants arranged ready for inoculation with eight test inocula of tomato spotted wilt.

Two independent Latin squares were planned, one on the lower and one on the upper leaves. For inoculation of test sample No. 1 all the plants with leaves ticketed 1 were withdrawn from the square and set along a bench convenient for inoculation. After inoculation they were replaced and the plants with leaves ticketed 2 removed, and so on. This was the procedure when time samples were being compared, but if it was necessary to compare, for example, eight different concentrations of electrolyte in the inoculum the eight dishes containing the inocula were all placed beside the operator and the plants brought along in order, each leaf being inoculated according to the ticket on it, from the appropriate dish.

Improvements were also made in the technique of inoculation. The ground-glass spatula previously described was used, but the blade of the spatula was increased in length to $1\frac{1}{2}$ in. (3.8 cm.) in order to fit comfortably across half of all except the largest leaves (Plate XXIII, fig. 2). The leaf to be inoculated was supported on a sponge rubber pad, about $\frac{3}{8}$ in. (9 mm.) in thickness, mounted on a piece of Bristol board. To prevent contaminations a fresh piece of newspaper was placed on the pad before

each inoculation. The inoculation was performed by dipping the spatula in the virus sample and lifting up with it enough liquid to cover the half-leaf surface easily, and drawing the spatula gently, firmly and fairly rapidly over the half-leaf from stem end to tip. No injury was visible on the leaf after inoculation except that the leaf hairs were pressed flat. Immediately after inoculation the leaf was washed with a stream of water from a wash-bottle.

Tests were made to determine whether there was any advantage in using a measured amount of inoculum for each inoculation as was done by Caldwell (2), but no such advantage was found, and the extra time required was a serious disadvantage. In one experiment with tobacco mosaic on *N. glutinosa* (32 replications) 0.1 ml. of inoculum was measured on to half of each leaf and inoculated with the spatula, and the other half was inoculated with excess of virus as lifted by the spatula (about 0.5–0.9 ml. according to the size of the leaf). The number of lesions on the sides inoculated with the measured amount of virus was 46.47 ± 2.93 and the number of lesions on the opposite sides was 46.16 ± 2.96 . There was thus no perceptible difference. In another experiment a measured 0.1 ml. was compared with a measured 0.3 ml. by Caldwell's finger-rubbing method, but the mean difference in numbers of lesions was not significant.

(3) *Pretreatment of test plants.*

It proved most convenient and in many cases necessary to perform all inoculations in the laboratory, and it was thought also that this practice might minimise possible differences due to temperature and humidity variation during sunny and cloudy periods in a greenhouse. Accordingly the plants were prepared for inoculation in a basement, the temperature of which remained relatively constant. It had previously been found that keeping plants for some days under reduced light conditions increased their "susceptibility" as measured by the numbers of lesions formed per unit area of leaf surface. The results of three experiments to investigate the course of this change are given in Table I.

The plants used were divided in the glasshouse into five groups as even as possible with respect to leaf area. One group was removed immediately to the basement, a second was taken the next day, and so on. The inoculation was carried out on all groups immediately the last one was brought in. When the lesion counts had been made the leaves were traced on brown paper and the area for each group calculated.

Table I.

Showing the effect of keeping test plants in the laboratory for various times before inoculation on the number of primary lesions produced by a given inoculum.

Time of transfer to laboratory	Time spent in laboratory prior to inoculation	No. of lesions*	No. of whole leaf replicates
Tomato spotted wilt on <i>N. tabacum</i> .			
5 p.m.	1 hour	1472†	16
	19 hours	1034†	
	(mostly night)		
	1½ days	2744	
	2¾ days	2675	
9.30 a.m.	15 min.	1568‡	20
	1 day	2312	
	2 days	2430	
	3 days	1941	
	4 days	2241	
Tobacco mosaic on <i>N. glutinosa</i> .			
9.30 a.m.	¾ hour	876‡	40
	1 day	2827	
	2 days	3217	
	3 days	3168	
	4 days	2731	

* The numbers of lesions given are the direct counts; correction for differences in area made no appreciable difference to the results.

† The difference between the first two totals of this experiment is not significant, but the difference between either of these two and either of the subsequent ones is significant.

‡ The first total is significantly different from the subsequent ones, but totals for 1, 2, 3 and 4 days in the laboratory are not significantly different from each other.

It will be seen that plants which had been indoors for 24 hours gave considerably more lesions with the same inoculum than did plants freshly transferred from the greenhouse. After the initial increase in susceptibility, however, the reaction of the plants remained fairly uniform. It was, therefore, made a general practice to bring the plants indoors 24 hours before they were required for inoculation.

III. EFFECT OF THE pH VALUE OF THE INOCULUM.

When investigating the effect of various chemicals on the activity of virus suspensions considerable changes in pH value and electrolyte content are often brought about if the additions are made to suspensions of the virus in water. Before valid conclusions can be drawn from such studies it is necessary to determine the effect of varying the pH value (at constant salt content) and of varying the salt content (at constant pH value) on the number of lesions produced by otherwise similar inocula.

In work previously reported (1) some preliminary tests were made in which the virus of tomato spotted wilt was treated with oxidising and reducing substances in such a way that the inocula must have varied greatly with respect to pH value, and the results of which were therefore open to question. This virus was selected for the first experiments on the effect of pH value.

After some preliminary tests to eliminate possible specific effects of buffering substances a composite buffer consisting of potassium hydrogen phthalate, potassium dihydrogen phosphate and boric acid was chosen as a stock from which all buffer solutions were subsequently prepared. This solution was 0.0533 molar with respect to each constituent. To prepare a solution of any desired pH value above 4 the appropriate volume of CO_2 -free NaOH solution was pipetted into 25 ml. of the stock buffer in a 100 ml. measuring flask and distilled water added to the mark. The resulting solutions were therefore 0.04 molar with respect to total salts. For solutions more acid than pH 4 the appropriate volume of 0.2 M HCl solution was added in place of the NaOH.

Previous experience had shown that one volume of juice in thirty of total volume gave a suitable inoculum from the point of view of the number of lesions developed. Inocula were therefore prepared by adding 1 part of expressed juice simultaneously to 29 parts of each of the test systems.

Precautions were taken to exclude oxygen from all inocula up to the time of inoculation. This was done by keeping the inocula in closed vessels through which a stream of purified nitrogen was bubbling. The test solutions themselves were freed of oxygen by the same means, and the only free oxygen in the system was that dissolved in the virus-containing juice, which would, however, soon be removed by the nitrogen stream. Nitrogen drawn from a cylinder of the commercial gas was passed in succession through several bubblers containing a 10 per cent. solution of $Na_2S_2O_4$ in 10 per cent. NaOH, a bubbler of oxygen-free water, a silica tube packed with short lengths of fine copper wire maintained at a temperature of $700^\circ C.$, another bubbler of oxygen-free water and finally through the inocula. To obtain an idea of the rate of any change taking place in the inocula samples were withdrawn at suitable intervals and inoculated on to the leaves of test plants (*N. tabacum* var. Blue Pryor) in accordance with a pre-arranged plan.

The results for a range of buffer solutions of different pH values are set out in Table II. Hydrogen and quinhydrone electrodes were used to determine the pH values of the inocula. Values obtained by the two

methods were in close agreement (within 0.01 pH unit) over the range where the quinhydrone electrode is applicable.

Table II.

Effect of the pH value of the inoculum on the number of primary lesions produced at various times. Tomato spotted wilt virus on N. tabacum.

Temperature 14°C.; juice concentration 1 in 30; composite buffer.

Exp. No.*	pH value	No. of lesions per leaf		
		5 min. approx.	$\frac{3}{4}$ hour	2½ hours
A	3.4†	0.01	0	0.01
	6.1	66	88	92
	7.7	86	97	103
B	4.3	0	0	0
	6.0	62	91	63
	8.5	78	97	83
C	5.1	52	0	0
	7.0	142	105	124
	9.2	176	83	51
D		4 min.	2 hours	5 hours
	6.0	72	67	33
	7.0	95	90	65
E		12 min.	2¼ hours	
	6.0	39	42	—
	11.0	0	0	—

* Exps. A-C. 9 × 9 Latin squares; nine whole-leaf replicates per treatment at each time.

Exp. D. Half-leaf comparisons on ten leaves per treatment at each time.

Exp. E. Half-leaf comparisons on eight leaves per treatment at each time.

† Two lesions only developed on twenty-seven leaves inoculated; probably contaminations.

On account of the design of the experiments and details of technique the data cannot be combined and used for the purpose of drawing curves to show the effects of pH on the longevity of the virus, but the results show quite clearly:

(1) That at and below a pH value of 5, and above a pH value of about 9.2, the inocula produced few lesions or none at all.

(2) Between pH values of 6 and 8.5 there appeared to be relatively small differences in the number of lesions produced by otherwise similar inocula, and at these pH values the inocula underwent very little change over a period of about 3 hours. Within these limits, however, significant differences did occur; *e.g.* in Exp. D the difference between virus at pH 6 and 7 was significant.

The necessity for keeping inocula of this virus within the pH range of 6–8 when investigating other properties is obvious. It is of interest to note that the pH value of juice freshly expressed from wilted tomato

plants, and the *pH* value of suspensions of the juice in water, often lie in the critical range between *pH* 5 and 6.

Some of the results previously reported⁽¹⁾ are readily explained in the light of these results. For example, the inactivating effect of a cysteine hydrochloride solution is easily understood, since the unbuffered solution must have had a *pH* value in the neighbourhood of 2. Later tests have shown that a suspension of the virus in a solution of cysteine buffered at *pH* 7 actually gives an inoculum which retains its activity longer than a parallel control without cysteine.

Work on similar lines with inocula prepared with the centrifuged juice from tobacco plants infected with ordinary tobacco mosaic showed that lesions resulted from all inocula between *pH* 1 and 11 with a fairly wide optimal range, but that below *pH* 1 and above *pH* 11 very few, if any, lesions resulted. More detailed studies on the relationship between *pH* value and the activity of these two viruses will be reported later.

IV. EFFECT OF ELECTROLYTE CONTENT ON THE NUMBER OF LESIONS PRODUCED.

Whatever the mode of entry of the virus into the leaf may be, the electrolyte content of the inoculum may quite conceivably affect the number of successful entries apart altogether from any effect on the virus itself. The experiments to be described were designed to determine the net effect of varying the salt concentration on the number of lesions produced by otherwise similar inocula. Two types of experiment were carried out. In one the concentration of buffering substances in the inoculum was varied, keeping the *pH* value constant; and in the other the concentration of buffering substances was constant throughout, but various amounts of other salts such as KCl and NaCl were added. This second type would give a measure of the effects (if any) due to the osmotic and similar action of added test systems, and also of the effects due to increased electrolyte content alone which might occur in more acid buffer solutions for the preparation of which HCl had to be added to the stock solution referred to previously.

Most of the tests were carried out with ordinary tobacco mosaic. For some of the earlier experiments the juice obtained by crushing the leaves of diseased plants in a mortar and straining through fine muslin was used directly, but in the later work and in the majority of the experiments reported here the juice so obtained was centrifuged at about 1500 g. for 1 hour to remove most of the suspended solid matter. In the earlier experiments a concentration of 1 volume of juice in a total

volume of 50 was used, but in later work with plants of somewhat higher virus content a juice concentration of 1 in 200 was employed. Where salts were used these were added to the solutions in the solid form or as concentrated solutions before making up to the final volume, and in all cases before adding the juice. The reagents throughout were of A.R. quality, in some cases further purified by recrystallisation. The results of typical experiments are set out in Table III.

Table III.

Effect of electrolyte concentration on the number of primary lesions produced by ordinary tobacco mosaic virus on N. glutinosa.

Exp.	Dilution and temp.	Nature of suspension medium	Number of lesions			Nature of experiment	pH value of inoculum
			Control	Test	Lesions on test — Lesions on control $\times 100$		
A		0.04 M composite buffer pH 7 (control) <i>versus</i> distilled water (test).					
	1/50 16.4° C.	—	1601	1016	63.4	Half-leaf comparisons 40 replicates	ca. 6
B		0.04 M composite buffer pH 7 (control) <i>versus</i> same buffer + various concentrations KCl and NaCl (test)					7.00
		KCl					
		0.01 M	1187	1231	103.7	Two 11 × 11	7.08
		0.05	937	1055	112.6	Latin squares	6.98
		0.10	926	975	105.3	22 half-leaf	—
		0.20	1085	955	86.0	replicates	—
	1/50	0.50	972	647	66.6	for each test system	6.75
		NaCl					
	17.5° C.	0.01 M	936	1084	115.9		6.99
		0.05	1126	1105	98.1		—
		0.10	1096	1220	111.3		—
		0.20	1035	921	89.0		6.79
		0.50	1019	699	68.6		6.63
		1.00	852	421	49.4		6.42
C		0.04 M composite buffer pH 7 (control) <i>versus</i> same buffer + KCl or other substances (test).					7.02
		KCl					
		0.01 M	370	535	144.6*	Randomised blocks	7.01
		0.05	382	461	120.7*	16 half-leaf replicates	6.95
		0.10	324	315	97.2	for each treatment	6.87
		0.20	307	356	116.0		6.85
		0.50	322	242	75.1*		6.78
		1.00	317	164	51.7**		6.68
		1.56	351	143	40.7**		6.62
	16.4° C. ca.	4.0 (satd.)	381	92	24.1**		6.62
		K ₂ SO ₄					
		0.01 N	274	231	90.6*		7.06
		1.00 N	292	125	42.8**		7.18
		Dextrose					
		0.01 M	256	236	111.7		7.00
		0.10	255	341	133.7		6.89
		0.78	270	224	83.0		6.04

Table III (cont.)

Effect of electrolyte concentration on the number of primary lesions produced by ordinary tobacco mosaic virus on N. glutinosa.

D	0.08 M composite buffer pH 7 (control) versus buffer solutions (pH 7) at various concentrations (test).					6.97
	Composite buffer					
	0.02 M	1141	927	81.2	Four 8 × 8	7.05
	0.04	1072	1080	100.7	Latin	7.03
	0.05	958	928	96.9	squares	7.00
1/200	0.08	1282	1230	95.9	32 half-leaf replicates for each test system	6.97
	Phosphate buffer					
	0.02 M	1110	1203	109.3		6.94
20° C.	0.04	1009	1086	107.6		6.92
	0.05	973	1125	115.6		6.91
	0.08	1012	1296	128.1		6.81
E	0.04 M phosphate buffer (pH 7) control versus phosphate buffer solutions (pH 7) at various concentrations (test)					6.86
	Dist. H ₂ O	532	342	64.3	Four 8 × 8	ca. 6
	Phosphate				Latin	
1/100	0.001 M	607	469	77.3	squares	6.61
	0.01	471	410	87.0	32 half-leaf	6.95
	0.02	481	440	91.5	replicates	6.95
	0.05	399	484	121.3	for each	6.86
	0.10	523	665	127.2	test system	6.83
	0.20	484	593	122.5		6.77
	0.50	545	309	56.7		6.66

* In section C significant differences are indicated by one asterisk when *P* is <0.05 and by two asterisks when *P* is <0.01. For sections B, D and E see the results of analyses of variance summarised in Table IV.

Table IV.

Statistical data for Table III.

Mean differences from control.

Table IIIB	Common control was 0.04 M composite buffer.					
	KCl					
	0.01 M	0.05 M	0.10 M	0.20 M	0.50 M	
	2.00	5.36	2.23	-5.91	-14.77	
	NaCl					
	0.01 M	0.05 M	0.10 M	0.20 M	0.50 M	1.00 M
	6.73	-0.95	5.64	-5.18	-14.55	-19.59

S.E. of each mean = 3.00. *P* = 0.01 when difference is 8.49.

S.E. of difference between any two means = 4.24. *P* = 0.01 when difference is 11.66.*

Table IIID	Common control was 0.08 M composite buffer.							
	Composite buffer				Phosphate buffer			
	0.02 M	0.04 M	0.05 M	0.08 M	0.02 M	0.04 M	0.05 M	0.08 M
	-6.69	0.25	-0.94	-1.63	2.90	2.41	4.75	8.88

S.E. of each mean = 2.19. *P* = 0.01 when difference is 6.02.

S.E. of difference between any two means = 3.10. *P* = 0.01 when difference is 8.52.*

Table IV (*cont.*).

Table III E

Common control was 0.04 *M* phosphate buffer.

Dist. H ₂ O	Phosphate buffer						
	0.001 <i>M</i>	0.01 <i>M</i>	0.02 <i>M</i>	0.05 <i>M</i>	0.10 <i>M</i>	0.20 <i>M</i>	0.50 <i>M</i>
-5.94	-4.31	-1.91	-1.28	2.66	4.44	3.41	-7.38

S.E. of each mean = 1.62. $P = 0.01$ when difference is 4.46.S.E. of difference between any two means = 2.29. $P = 0.01$ when difference is 6.30.*

* In arriving at this and similar values where the number of degrees of freedom was greater than 30, the value of t for $n=30$ was used rather than interpolating to the actual number of degrees of freedom. Any difference between these and the true values is negligible and in any case tends to make the tests for significance more stringent.

The results of a statistical examination of some of the figures set out in Table III are summarised in Table IV. For the purpose of these analyses the difference between the number of lesions on the control half of each leaf and the test half was taken as the basis of comparison so that the treatment means given in Table IV all refer to a mean difference between test (treatment) and its control. In each experiment one inoculum buffered at pH 7 was used as a common control for all test systems.

It will be seen that the design of the experiments is such that the means given above may be compared with the appropriate standard errors in order to estimate the significance of any difference between each individual treatment and its control, and also enables an inter-comparison between any two means in the same experiment to be made on the same basis.

The following main conclusions may be drawn from these results:

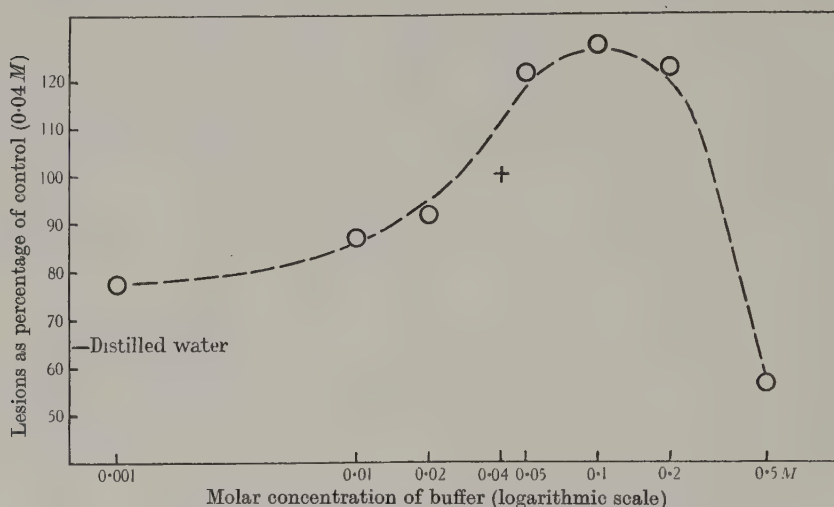
(1) Significantly more lesions were produced by suspensions of infective juice in 0.04 *M* potassium phosphate buffer at pH 7 and in 0.04 *M* phosphate-phthallate-borate buffer at pH 7 than by similarly prepared suspensions in distilled water. The difference was greater than could be accounted for by difference in pH value.

(2) The effect of the phosphate buffer increased with concentration to an optimum that, in the two experiments quoted, lay between the limits 0.05 *M* and 0.2 *M*. With a further increase in concentration there was a sharp drop in the numbers of lesions produced (Text-fig. 1).

(3) Virus in the presence of the phosphate-phthallate-borate buffer produced more lesions at a concentration of 0.04 *M* and 0.08 *M* than at a concentration of 0.02 *M*.

(4) Taken as a whole over the range 0.02–0.08 *M*, the virus in the presence of the composite buffer produced fewer lesions than in the presence of the phosphate buffer.

(5) In the experiments (Table III B and C) in which various electrolytes and dextrose were added to infectious juice buffered at pH 7 with 0.04 *M* composite buffer the lower concentrations had little effect, though in some cases the numbers of lesions were significantly raised. These lower concentrations were of the same order as those of the composite and phosphate buffers that produced the maximum number of lesions. Above a certain concentration (roughly 0.2 *M*) a continuous fall in the number of lesions was produced by increasing concentrations of all the substances tested.



Text-fig. 1. Showing the effect of increasing concentrations of potassium phosphate buffer at pH 7 on the numbers of lesions produced by tobacco mosaic virus on *Nicotiana glutinosa*.

(6) Sodium and potassium chloride at equal concentration depressed the number of lesions to the same degree.

After obtaining these results the electrolyte content of inocula in subsequent experiments was adjusted so that it fell within the optimum range for the particular buffer used.

In the case of the virus of tomato spotted wilt comparisons such as those described above are complicated by the rapid inactivation of the inocula during inoculation, thus making experimental error high. However, preliminary experiments have demonstrated the absence of any effect due to the presence of electrolyte over the concentration range employed in work reported in this paper.

V. EFFECT OF OXIDISING AND REDUCING AGENTS.

Previous work⁽¹⁾ had shown that the addition of certain oxidising and reducing agents to aqueous suspensions containing spotted wilt virus caused a falling off in the number of lesions produced by subsequent inoculation. Sodium sulphite, on the other hand, exerted a marked preservative action. At the time, this action of sodium sulphite was ascribed to the reducing properties of this substance, and the inactivating effect of certain of the former class to their oxidising properties.

On the basis of the results described in this paper it is evident that these effects could not have been due to the higher electrolyte content of the test inocula, but there still remained the possibility that changes in pH value induced in the test inocula by the added substances may have been responsible for part at any rate of the observed effects. More detailed work on this subject will be published later, but for our present purpose we wished to determine whether the addition of such substances to inocula would result in a change in the number of lesions produced when effects due to other controllable factors were eliminated.

Inocula were prepared along the lines already described when dealing with the pH value of inocula. Buffer solutions (0.04 M) were prepared so that the final pH value, after adding the test substances, and finally the juice, would be in the neighbourhood of pH 7 (which falls in the optimum range). In no case was the final concentration of added substance greater than 0.01 M . Inert electrolytes added in amounts greater than this did not affect the number of lesions produced, so that it was considered unnecessary to adjust for the small difference in electrolyte content between the test and control inocula. Aliquots of test and control suspensions were withdrawn after various time intervals and inoculated on to tobacco plants by the usual methods. Under these conditions H_2O_2 gave results in accordance with the previous findings. In addition the presence of $KMnO_4$ (0.01 M and 0.001 M), $K_3Fe(CN)_6$ (0.005 M) and benzo-quinone (0.005 M) resulted in a rapid reduction in the number of lesions as compared with controls. The test inocula produced some lesions within 5 min. of preparation, but within half an hour they produced none. The corresponding controls gave many more lesions at the outset and remained active for some hours.

The preservative action of Na_2SO_3 previously recorded has been confirmed under these conditions. The effect of Na_2SO_3 in changing an aqueous suspension to a more favourable pH value was therefore only of minor importance, although it must have had some effect. Attempts to

"reactivate", by adding Na_2SO_3 , virus which had been inactivated in air gave negative results under these controlled conditions. In experiments previously reported⁽¹⁾ there was an increase in the number of lesions relative to the control as a result of adding Na_2SO_3 to a partially inactivated suspension of infective juice in water. Probably the increase should be ascribed to other causes than reactivation, since in any case the *pH* values of the inocula, although they were not known, must have been different (that for the aqueous suspension probably falling within the critical *pH* range).

A 0.01 *M* solution of $\text{Na}_2\text{S}_2\text{O}_4$ (buffered as in all these tests) was found to act in the same way as the sulphite. As mentioned before, a solution of cysteine hydrochloride (0.01 *M*) adjusted to *pH* 7 gave an inoculum initially equal in strength to the control, which did not fall off as rapidly. In order to decide whether the protective effect of these substances was associated with their reducing properties or with some other property which they might possess in common, the effect of storing an inoculum in a stream of hydrogen in the presence of platinised platinum gauze was tested against a control through which only hydrogen was passed. The same preservative effect was observed, so that in the light of our present knowledge it is reasonable to assume that the action of these preservative substances is due to their common property as reducing agents.

VI. CONCLUSION.

It should be stated that the effects reported in this paper may or may not be directly concerned with the virus itself. This problem of differentiating between direct and indirect effect is by no means a simple one. In the meantime, however, it is obvious that in all quantitative work with plant viruses, whether by the primary lesion method or any other, greater attention should be paid to the *pH* value and electrolyte content of the inocula if misleading conclusions are to be avoided. Furthermore, when dealing with inocula which undergo rapid inactivation on exposure to air the possibility of oxidative processes operating in the inocula should be examined and minimised when other properties are under investigation. It is clear, for example, that a redetermination of the thermal death-point of the virus of tomato spotted wilt is required at a *pH* value within the optimum range and under conditions which will minimise inactivating processes of an oxidative nature. Filtration and other experiments, too, may be expected to give different results if carried out under conditions found to be favourable for the maintenance of activity of the virus.

VII. SUMMARY.

The arrangement of experiments for intercomparison of a number of virus samples is discussed and the use of randomised blocks or Latin squares, combined in certain cases with half-leaf comparisons between all samples or against a standard, is recommended.

Improvements in the ground-glass spatula method of inoculation have been suggested.

Provided there is sufficient inoculum to cover the leaf when the spatula is rubbed over it, the amount of inoculum makes no difference to the number of lesions produced.

The conditions to which test plants are subjected shortly before inoculation were found to influence the number of lesions produced by a given inoculum.

The number of lesions produced by otherwise similar inocula is influenced by their *pH* value and electrolyte content. The optimum *pH* range for the virus of tomato spotted wilt is very limited (roughly *pH* 6.0–8.5). Tobacco mosaic virus in a potassium phosphate buffer at a *pH* value about 7 produced the maximum number of lesions in the concentration range 0.05–0.2*M*. It is recommended that for most quantitative work viruses should be buffered at a definite *pH* value.

When effects due to varying *pH* value and electrolyte content were excluded, the virus of tomato spotted wilt was still found to be inactivated by certain oxidising agents and preserved by certain reducing agents.

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EXPLANATION OF PLATE XXIII.

Fig. 1. Tobacco plants arranged ready for comparison of eight samples of tomato spotted wilt virus. Two independent Latin squares were planned, one for the lower leaves and one for the upper leaves. For inoculation of sample No. 1 all plants bearing a leaf ticketed "1" were withdrawn and set along a bench convenient for inoculation, being replaced in the square afterwards. Similarly for the remaining samples.

Fig. 2. The technique of inoculation, showing elongated ground-glass spatula and sponge-rubber pad covered with a fresh square of newspaper for each inoculation.

(Received March 15th, 1935.)



Fig. 1.



Fig. 2.

SAMUEL, BEST AND BALD.—FURTHER STUDIES ON QUANTITATIVE METHODS WITH TWO PLANT VIRUSES (pp. 508-524).

INSECT INJURY SIMULATING FUNGAL ATTACK ON PLANTS

A STEM CANKER, AN ANGULAR SPOT, A FRUIT SCAB AND
A FRUIT ROT OF MANGOES CAUSED BY *HELOPELTIS*
BERGROTHI REUT. (CAPSIDAE)

By R. LEACH, B.A., A.I.C.T.A.

(*Mycologist, Department of Agriculture, Nyasaland.*)

(With Plates XXIV and XXV and 3 Diagrams in the Text.)

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INTRODUCTION.

IN 1933 Leach and Smee(4) published a paper claiming that a stem canker of tea, which until then was considered to be due to fungus parasitism, was in reality caused by the capsid bug, *Helopeltis bergrothi* Reut. Steyaert and Vrydagh(6), also in 1933, published an account of a stem canker, leaf spot and anthracnose of cotton caused by the same insect in the Belgian Congo. Moreau(5), again in 1933, even claimed that the disease of cotton known as "black-arm" is either a direct result of leaf punctures by *H. bergrothi* or possibly due to a virus disease transmitted by it".

This paper gives further evidence that the types of diseases described can certainly be caused on mangoes by the capsid bug, *H. bergrothi*,

although the symptoms are like the attack on the host by a fungus or bacterium.

Although no reference has been found to diseases of mangoes similar to those described in this paper, it is possible that the results of this work may be of direct assistance to those countries where, mangoes being of economic importance, similar diseases may occur. The scab of the fruit might render them unmarketable, while the fruit rot might cause a considerable loss of crop.

CAUSE OF THE DISEASES.

Although suspicions were aroused that stem canker was caused by a sucking insect, it was not until July 1934 that a nymph of *H. bergrothi* was found in the act of forming a canker lesion on a young mango stem. Bagging experiments with mosquito-net "sleeves" proved that the insect caused the stem canker, angular leaf spot, fruit scab and fruit rot. The primary lesions are usually recognisable before the insect has withdrawn its stylets from the sucked tissue. The adults and nymphs feed readily on the young stems, leaves and fruit of the mango tree.

No special attention has been paid to the relative activities of the different stages of the insect. The experimental work has been carried out to study the morbid anatomy of the disease from the time that the insect stops feeding. No organism has been seen or isolated from the freshly affected tissue, and pieces of this tissue applied to the cut or uncut surface of healthy young stems, leaves and fruits have not transmitted the diseases. The feeding habits and life history of the insect on tea in Nyasaland have been described by Leach and Smee (4). On mango oviposition occurs on the young stem, eggs being readily found near fresh canker lesions. No eggs have been seen on the fruit.

TECHNIQUE.

(i) *Macroscopic examination of lesions.* This was made in the field after bagging insects on to the stems, leaves and fruit for one hour only in the early morning.

(ii) *Microscopic examination of lesions.* Specimens were fixed in formalin-acetic-alcohol and cut with a Spencer hand microtome. Safranin and Delafield's haematoxylin was the double stain employed.

(iii) *Examination of stylets in situ.* The same method was used as that employed by Leach and Smee (4). Adults and nymphs were used in the laboratory for forming stem cankers and angular leaf-spot lesions. For the formation of fruit-scab and fruit-rot lesions the oldest nymphs were

used because it was necessary to place the insect on the fruit on the tree, lesions not developing normally in the laboratory. This is probably due to the drainage of a large quantity of resin from the fruit stalk when cut. Owing to the mass of resin ducts in the fruit skin, this loss of resin may cause "drying" of the skin preventing the normal development of the lesions.

PATH OF STYLETS IN THE HOST PLANT.

Experiments were carried out to trace the path of the stylets of the insect when feeding in the stem, leaf and fruit. Twenty insects were fixed *in situ* in each case.

Table I.

Bottom 4 in. of stem of young flush.

Stylets found in ...	Cortex	Pericycle paren- chyma	Resin fibre ring	Phloem	Xylem	Pith
Canker lesions formed	0	16	0	0	1	0
No canker lesions	1	0	0	0	2	0

Table I shows that cankers are formed in nearly every case when the stylets end in the pericycle parenchyma. The one exception is probably due to movement of the stylets after first being situated in the pericycle.

Table II.

Top 2 in. of stem of young flush.

Stylets found in ...	Cortex	Pericycle paren- chyma	Resin fibre ring	Phloem	Xylem	Pith
Canker lesions formed	0	6	3	0	5	4
No lesions formed	1	0	0	0	1	0

Table II shows that in very young stems, where little or no secondary thickening has commenced, the stylets are found in many tissues other than the pericycle parenchyma when cankers are formed. This is probably due to the insect being able to move its stylets more freely in the soft and as yet unligified tissue. The table explains the anomalous formation of hyperplasy associated with old cankers (*a*) in the resin fibre ring, (*b*) in the pith, and (*c*) to a slight extent in the xylem.

Table III.

*Position of the stylets in relation to the formation of
fruit scab and fruit rot.*

Stylets found in ...	Outer skin	Middle skin	Inner skin
Scabs formed	1	15	0
Rot lesions formed	—	2	2

Table III shows that fruit-scab lesions are formed when the stylets penetrate no farther than the middle skin (Diagrams I, II and III). Rot lesions are formed when the stylets reach the inner skin. The two exceptions probably occurred owing to the stylets being withdrawn when the insect was killed. All four insects causing cankers had their stylets deeply inserted at the time the wax was applied.

ANATOMY OF MANGO FRUIT.

Since the formation of the scab or rot of the fruit depends on the depth to which the stylets penetrate, the structure of the skin must play an important part in the development of these two diseases. For this reason a short account of the structure of the mango fruit is given here.

The fruit is a drupe (Diagrams I, II and III); it consists of a seed surrounded by a pericarp which is differentiated into a succulent exocarp and a gradually hardening endocarp. The succulent exocarp is divided into two regions, (*a*) the skin and (*b*) the flesh of the fruit.

(*a*) The skin consists of an epidermis, with thick outer walls, beneath which is parenchyma interspersed with resin ducts. It is apparently the density of these ducts and the size of the parenchymatous cells which controls the development of the lesion. This parenchyma of the skin is divided into three regions: (i) a thin (150μ) outer region composed of small, round, fairly thick-walled cells containing chloroplasts and interspersed with few resin ducts, (ii) a middle region (700μ) composed of thin-walled, round cells, increasing in size towards the next region, containing few chloroplasts but interspersed with numerous small resin ducts, and (iii) an inner region (700μ) composed of larger, thin-walled cells increasing in size towards the flesh and interspersed with a few widely spaced large resin ducts (Diagrams I, II and III).

(*b*) The flesh of the fruit is composed of large, watery, thin-walled cells, loosely jointed and containing a number of minute vascular bundles but only a few resin ducts next to the skin.

The endocarp is soft in the unripe fruit and is similar in structure to the skin of the exocarp, its three regions bearing the same relationship with the flesh.

The resin ducts in the fruit are secretory passages forming a "branched anastomosing system of tubes" (3). They are not surrounded by fibres in the skin. Except the smallest ducts, however, all have minute vascular bundles associated with them in a "characteristic and constant manner".

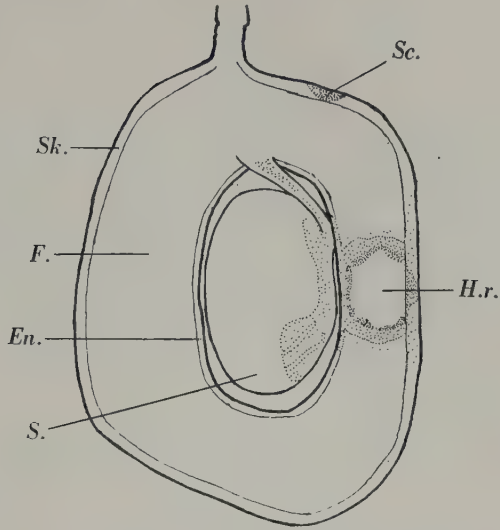
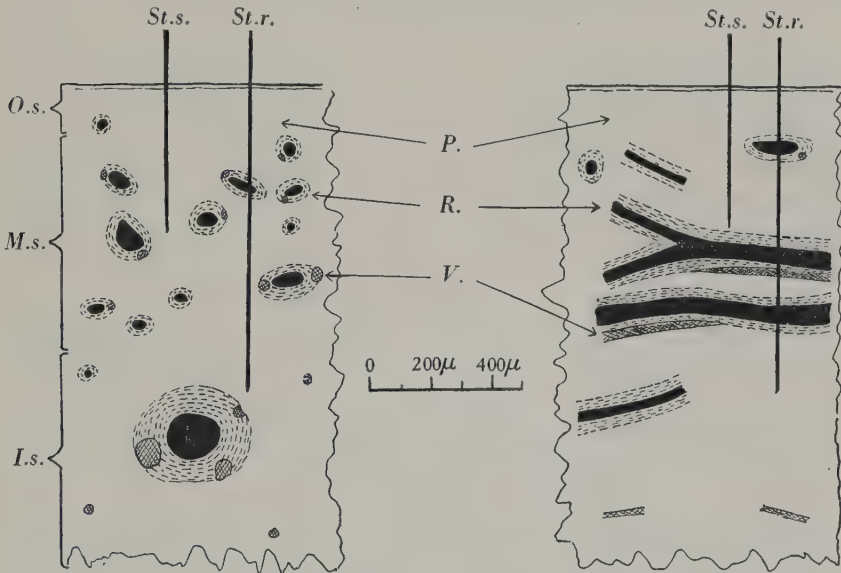


Diagram I. Longitudinal section fruit showing initial scab (*Sc.*) and rot (*H.r.*) lesions.



Drawn from camera lucida (except stylets)

Diagram II. Transverse section of skin of fruit.

Diagram III. Longitudinal section of skin of fruit.

Sk. skin; *F.* flesh; *En.* endocarp; *S.* seed; *O.s.* outer skin; *M.s.* middle skin; *I.s.* inner skin; *P.* parenchyma; *R.* resin duct; *V.* vascular bundle; *Sc.* scab lesion; *H.r.* hollow rot of rot lesion; *St.s.* stylets forming scab; *St.r.* stylets forming rot. Discoloured areas shaded.

DEVELOPMENT OF STEM CANKER.

Macroscopic.

The lesions are first seen as water-soaked, green, oblong-ovate lesions in the otherwise copper-coloured stem. The size of the lesions averages 15 mm. long by 3 mm. broad. Within twenty-four hours the lesions are slightly sunken and turn yellow-brown at the edges. Longitudinal ridges sometimes occur, being caused by the collapse of soft parenchymatous tissue between fibrous rings round the longitudinal resin ducts. The lesions later turn completely yellow-brown and become more sunken. It is at this time that the lesions may turn black, starting from the centre (the point of entry of the stylets).

After two weeks the cankers are no longer sunken. Occasionally they may be deeply cracked, causing open wounds in the stem.

The cankers may be (i) completely black with fungus fructifications visible on the surface, (ii) partially blackened with slightly raised, smooth, yellow-brown edges (Plate XXIV, fig. 1) or (iii) slightly raised, smooth, yellow-brown with no blackening.

A bead of sap is often exuded from the puncture of a fresh lesion. This forms an ideal point of entry for bacteria and fungi, as it may remain for twenty-four hours in damp weather. The blackening of the lesions is undoubtedly due to the invasion of weakened cortical tissue by fungi and bacteria. The number of black cankers is approximately 90 per cent. in damp weather and 30 per cent. in dry weather. The blackening is confined to the area originally affected and does not spread at all.

Microscopic.

The insect generally feeds in the pericycle parenchyma; the stylets may, however, penetrate to various depths in the stem. Large resin ducts are surrounded by lignified fibres. These fibres, once they become lignified, probably act as a barrier to the stylets as they are situated in an almost continuous ring outside the phloem.

The cells next to the cortex are the first to be affected; they start to collapse twenty-four hours after the insertion of the stylets (Plate XXV, fig. 1). The next cells to collapse are those which adjoin the fibre ring of the resin ducts (Plate XXV, fig. 2). During the next few days further groups of cells may collapse between the two initially affected areas. In the youngest stems, cells may collapse in the unlignified fibre rings, in the phloem, in the xylem parenchyma and in the pith (Plate XXV, fig. 3).

After a week to ten days hyperplasy originates from unaffected cells in or on the border of the affected regions. This development of hyperplastic parenchyma usually commences close to a resin fibre ring (Plate XXV, fig. 4).

In the fully developed canker the hyperplastic growth causes a slight swelling on the stem, due to the pushing out of the cortex. The cortex may become necrotic and blackened by the invasion of weak parasitic fungi (*Colletotrichum* spp. and *Phoma* spp.) and bacteria, or it may remain healthy throughout the development of the canker, in which case it does not collapse (Plate XXV, fig. 5).

Hyperplasy of the pith cells is often considerable; if it occurs in the resin fibre ring the latter is broken up into small groups of fibre cells (well shown by staining with phloroglucin).

The cambium is rarely affected, but when it is, the canker may form an open wound with the development of wound wood, as in gnarled stem canker of tea.

DEVELOPMENT OF ANGULAR LEAF SPOT.

Macroscopic.

The insect feeds on the copper-coloured young leaves. The sucked area, a faint spot which loses its anthocyan pigment, is visible directly the insect stops feeding. It is white when held against the light, but appears olive-green when seen against a dark background. The spots, being bounded by veins, are more or less angular. Their size varies from 1 to 4 mm. in diameter.

The spots do not change colour quickly; they gradually become a light yellow-brown with slightly darker edges in four to five days. In dry weather they reach their final colour of light brown with dark brown edges after two weeks. In wet weather the discoloration of the tissues is more rapid and the spots soon become dark brown or black probably due to the invasion of the necrotic tissue by fungi or bacteria (Plate XXIV, fig. 2).

The spots usually remain papery in the centre, but sometimes they fall out and give a shot-hole appearance in the older leaves.

The insect feeds anywhere on the leaf; it feeds frequently at the edge of the veins. Feeding along the midrib and petiole forms elongated lesions which cause considerable curling and puckering of the leaf.

Microscopic.

Without the facilities for imbedding, no examination of the stylets fixed *in situ* could be made, because the latter are so easily displaced. As far as could be ascertained, the stylets end in the palisade parenchyma.

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The cells are so soft that they collapse quickly. The affected tissue is bordered by the largest veins, the smallest veins being incorporated in the necrotic tissue.

Hyperplasy is negligible on the edge of the necrotic tissue, except when the latter is next to the midrib, in which case a distinct development of hyperplastic parenchyma is formed outside the pericycle fibre ring of the midrib.

DEVELOPMENT OF FRUIT SCAB.

Macroscopic.

The insect feeds on all sizes of unripe fruit, but usually on the younger ones.

At first the lesion is a round, water-soaked area, 2–5 mm. in diameter, slightly darker green than the healthy tissue against which it is sharply defined (Plate XXIV, fig. 4a). Within twenty-four hours the lesion becomes sunken and turns light brown in the centre, often exuding small, sticky, orange-coloured beads of resin. During the next two days the lesion gradually becomes a well-defined black spot remaining the same size as when it was first formed.

This blackening is due to some property of the resin in the skin because, unlike the canker and angular leaf spot lesions, the scab lesions *always* turn black. The spot is seen to be confined to the skin on cutting across the fruit (Plate XXIV, fig. 5 and Diagram I). These black spots dry and harden during subsequent growth of the fruit. They are separated from the healthy tissue by a light-coloured, sunken, corky ring varying in breadth up to 5 mm. (Plate XXIV, fig. 3). The scabs may be situated close together but they are usually separated from each other by the cork rings. The scabs often split into smaller portions during growth of the fruit.

Heavy scabbing of the smaller fruit usually leads to the early falling of the fruit.

Microscopic.

The stylets enter only as far as the middle of the skin (Diagrams II and III). The initially affected area consists of small parenchymatous cells broken up by numerous resin ducts and their associated vascular bundles. It is the writer's opinion that the toxin, injected through the stylets, can only diffuse slowly through this tissue owing to the small size of the cells and to the mass of resin ducts and small vascular bundles. The toxin is thus unable to penetrate as far as the large thin-walled cells

of the flesh before the plant has time to react against its progress (cf. fruit rot).

The affected area does not discolour for twelve hours, when the parenchyma turns brown; the sheathing layers of the resin ducts become discoloured and the secretory cells and resinous fluid turn brown after thirty-six hours. The cells start to collapse after twenty-four hours. The development of hyperplastic parenchyma starts on the border of the affected region next to the flesh in four hours. The parenchyma and resin ducts are then crushed in the manner shown in Plate XXV, fig. 6. This photograph shows the last stage of scab formation. A layer of phellogen which gives rise to cork on the outside is formed immediately below the scab. When growth of the fruit causes the scab to split away from the healthy tissue, this layer of cork forms a ring round the scab (Plate XXV, fig. 6).

DEVELOPMENT OF FRUIT ROT.

Macroscopic.

The initial symptom may not be visible for twelve hours after insertion of the stylets. It then appears as a small, irregular, dark green, water-soaked area gradually increasing in size and spreading over the surface of the fruit as shown in Plate XXIV, fig. 4*b*. This symptom of the disease is, however, usually hidden at first owing to the fruit rot lesion being associated with a fruit scab lesion, the insect having fed first in the middle skin and then in the inner skin or *vice versa*. The first sign of rot appears then after twelve to twenty-four hours and is apparent as an irregular, dark green, water-soaked zone extending round the light brown circular scab lesion.

If the fruit is cut across through the middle of one of these lesions, the following zones of affected tissue are recognisable (Plate XXIV, fig. 5 and Diagram 1).

(1) The affected portion of the skin is water-soaked and green except for the light brown discoloration round where the stylets entered.

(2) A small hollow, containing a clear brown liquid, below the skin; this hollow rotted area varies in breadth from 0.5 to 1.0 cm. and may extend as far as the endocarp.

(3) A water-soaked light brown region in the flesh on each side of the rotted hollow area varying in thickness from 1 to 5 mm.

(4) A brown discoloration of the endocarp and of the seed if the rotted area extends as far as the endocarp (not shown in Plate XXIV, fig. 5).

On cutting across the fruit for examination of the stylets *in situ* it was noticed that the hollow rot commences very rapidly. The hollow may be 2 mm. in diameter in two hours; the whole internal lesion is light brown in colour by that time.

The discoloration spreads rapidly in the skin. Three-quarters of the fruit's surface may be affected within three days. Numerous small, sticky beads of resin generally appear on the surface. If the fruit is cut across at this stage the affected skin is brown, as may also be the endocarp, while the white flesh has turned a dull yellow colour. The fruit usually falls in this condition. The disease is commonest on the very small fruit which may hang on the tree for some time looking like large raisins. Any larger fruits which remain on the tree shrivel and become copper to russet brown in colour (Plate XXIV, fig. 6).

Microscopic.

When the stylets penetrate deeper into the skin than usual (Diagrams II and III) the toxin is probably able to diffuse more quickly through the larger parenchymatous cells of the inner skin and has only a few resin ducts to impede its progress towards the large, thin-walled cells of the flesh. The plant is thus probably unable to react in time to prevent the toxin spreading rapidly, with the result that the lesion is much larger than that of the affected scab.

This disease is difficult to follow microscopically. The first cells to be affected are rapidly discoloured and then become so soft that sectioning of the tissue is difficult. Hyperplasy commences on the edge of the affected region in two hours. The toxin breaks down the first hyperplastic cells to be formed.

The contents of the affected cells of the flesh turn brown but the cells do not collapse until the fruit starts to dry. Similarly the skin cells do not collapse for some time although the contents of the parenchymatous cells and the resinous fluid are discoloured.

The spread of the initial rot lesion to include the whole fruit is very interesting, but difficult to explain. It is noticeable that the regions supplied with resin ducts are the ones in which the brown discoloration spreads. The clear liquid of the hollow rotted area *possibly* includes the toxin in solution. This solution is perhaps distributed via the resin ducts in the inner skin and causes the discoloration and necrosis of the parenchymatous cells to which it is distributed. If the toxin were carried in the vascular bundles of the fruit, the flesh would be discoloured as soon as any other tissue, which is not the case.

CONTROL MEASURES FOR THE FOUR DISEASES.

As the diseases are shown to be due entirely to the feeding of the capsid bug, *Helopeltis bergrothi*, on the stem, leaf and fruit of the mango tree, it is obvious that all measures for control must aim at frustrating attack by this insect.

The principles advocated for the control of this and another species of *Helopeltis* on tea may be observed. Reference to the work of Leach and Smee (4) in Nyasaland and Andrews (1) in India can be made for this purpose.

DISCUSSION.

It is necessary for the advisory mycologist to make a rapid diagnosis of the cause of any disease put before him and to formulate control measures as quickly as possible. Butler (2) states that "the diagnosis of a disease means the discovery of its identity or nature. Symptoms are the outward signs by which a diagnosis is assisted". Under the headings of symptoms he includes among others "canker", "leaf-spotting", "scab" and "rotting".

The symptoms of the four diseases of mango described in this paper are similar to many fungus or bacterial diseases described by Butler under the above headings. It is possible therefore that symptoms such as these may start investigations along wrong lines, leading to the waste of much time and energy in studying the aetiology of diseases and may possibly lead to unnecessary expense in attempting to combat the diseases.

The symptoms of these four types of disease caused by sucking insects tend to differ from those of fungus or bacterial origin in the two following main points:

(1) The lesions are produced quickly by the insect, *i.e.* a stem, leaf or fruit may be covered with lesions of an appreciable size in one night—quick work for a fungus or bacterium!

(2) The lesions usually do not increase in size from the time they are first visible. Fruit rot of mangoes is an interesting and important exception; the rot spreads through the fruit in a manner extraordinarily like that of a fungus or bacterium.

Gnarled stem canker of tea, four diseases of mango and, according to Steyaert and Vrydagh (6), similar diseases of cotton have been shown to be due to the sucking of the same species of capsid bug (*Helopeltis*

bergrothi, Reut.).¹ It seems highly probable that there are more insects of the same type which are capable of causing similar diseases of other crops in both temperate and tropical countries. The present work has been carried out in close co-operation with the Entomologist, Department of Agriculture, Nyasaland. It is the writer's opinion that such co-operative work between applied entomologists and applied mycologists would lead to the diagnosis of more diseases of these types.

SUMMARY.

1. Four different types of disease of the mango tree are shown to be caused by the capsid bug, *Helopeltis bergrothi* Reut.
2. Symptoms of a stem canker, an angular leaf spot, a fruit scab and a fruit rot of mangoes are described.
3. A description of the morbid anatomy of these diseases is given.
4. References to control measures advocated against *H. bergrothi* are cited.
5. A discussion of the results shows the possibility of the importance of further co-operative work between entomologists and mycologists in the study of plant diseases.

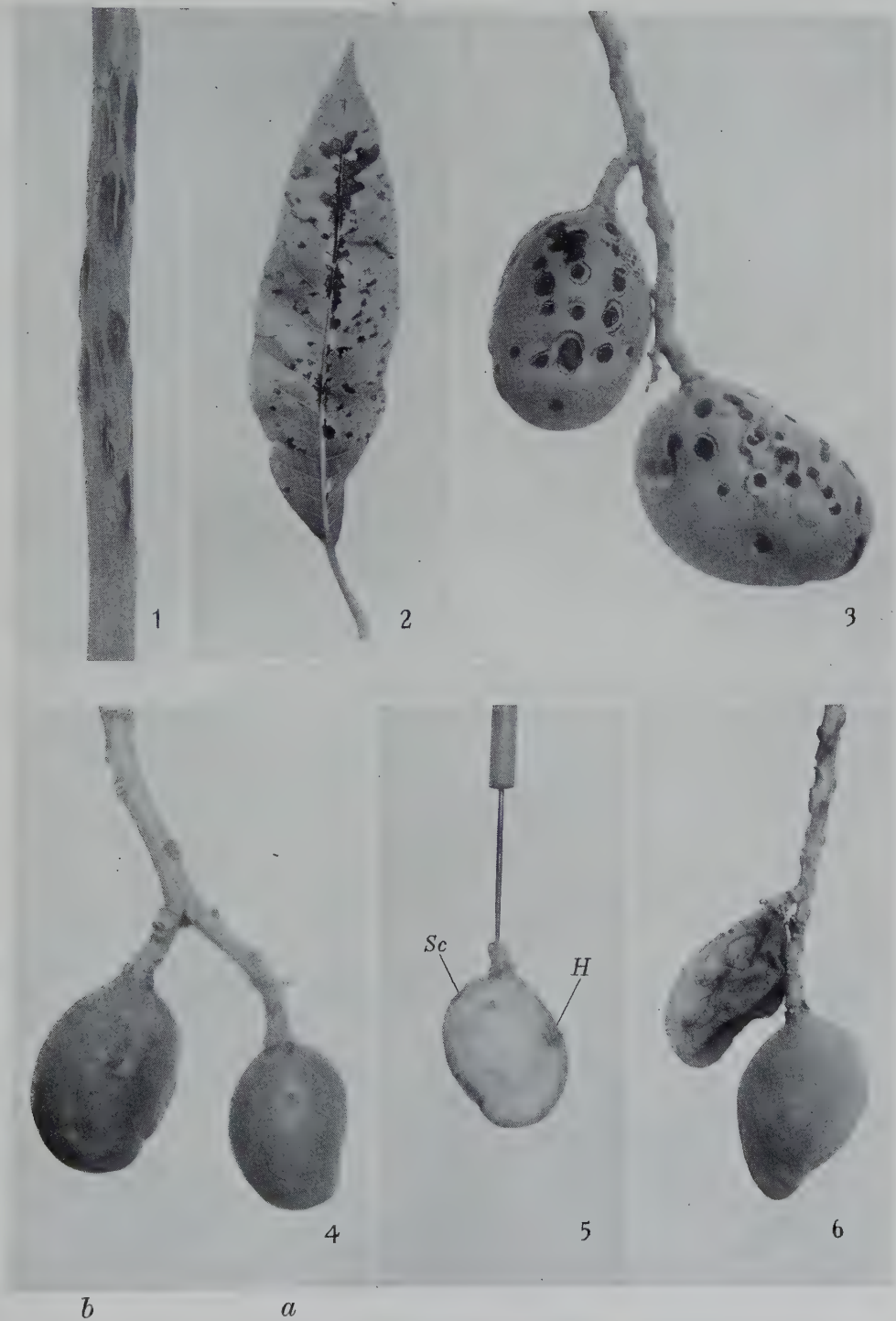
ACKNOWLEDGMENTS.

I wish to acknowledge with thanks the assistance and helpful criticisms of Capt. C. Smee, Entomologist, Department of Agriculture, Nyasaland. I am indebted to Dr W. Small, Director of Agriculture, Nyasaland, for affording me the facilities to carry out this work, to Dr E. J. Butler, Director of the Imperial Mycological Institute, Kew, for some helpful advice and criticism and to Mr L. W. Stewart, Eldorado Estate, Mlanje, for the kind loan of a camera.

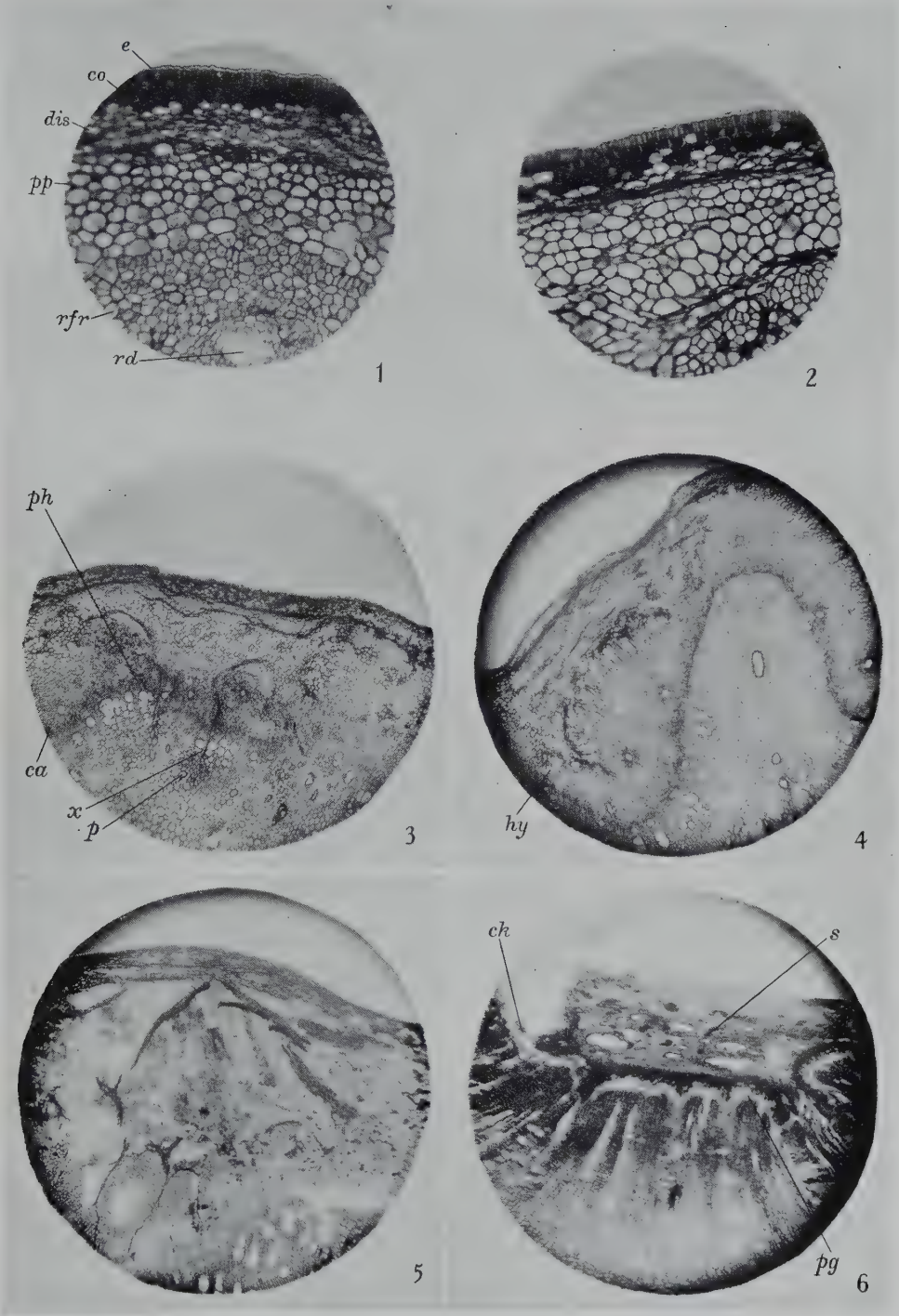
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¹ Since writing this paper a black scab of avocado fruit, sent in for examination from an estate, has been proved to be caused by *Helopeltis bergrothi*. The symptoms are very similar to those of mango scab. A white crystalline exudation from the scabs was thought by the planter to be a fungus. Last year he sprayed with Bordeaux mixture to combat the "fungus". The treatment, of course, met with no success.



LEACH.—INSECT INJURY SIMULATING FUNGAL ATTACK ON PLANTS (pp. 525–537).



LEACH.—INSECT INJURY SIMULATING FUNGAL ATTACK ON PLANTS (pp. 525-537).

EXPLANATION OF PLATES XXIV AND XXV

PLATE XXIV.

- Fig. 1. Stem canker of mango. $\times \frac{3}{4}$.
 Fig. 2. Angular leaf spot of mango. $\times \frac{3}{4}$.
 Fig. 3. Fruit scab of mango. $\times \frac{3}{4}$.
 Fig. 4. First stage of (a) fruit scab and (b) fruit rot of mango. $\times \frac{3}{4}$.
 Fig. 5. Mango fruit cut longitudinally showing first stage of rot. *H*, hollow rotted area; *Sc*, scab lesion. $\times \frac{3}{4}$.
 Fig. 6. Fruit rot of mango. Rotted fruit attached above healthy fruit. Original lesion visible. $\times \frac{3}{4}$.

PLATE XXV.

- Fig. 1. Development of canker. I. Twenty-four hours after insertion of stylets. *e*, epidermis; *co*, cortex (stained deeply, not collapsed); *pp*, pericycle parenchyma; *rd*, resin duct; *rfr*, resin fibre ring; *dis*, pericycle parenchyma starting to collapse. $\times 95$.
 Fig. 2. Development of canker. II. Forty-eight hours after feeding. Pericycle parenchyma cells next to resin fibre ring collapsed. $\times 95$.
 Fig. 3. Development of canker. III. One week after feeding. Very young stem. Cells in pericycle parenchyma, resin fibre ring, (*ph*) phloem, (*ca*) cambium, (*x*) xylem parenchyma and (*p*) pith collapsed. $\times 28$.
 Fig. 4. Development of canker. IV. Two weeks after feeding. Hyperplastic parenchyma (*hy*) developing in pericycle on edge of resin fibre ring. (Cortex folded over, not collapsed.) $\times 28$.
 Fig. 5. Development of canker. V. Two months after feeding. Extensive development of hyperplastic parenchyma. Cortex healthy but pushed out by hyperplastic development to form a swelling on the stem. $\times 28$.
 Fig. 6. Fully developed fruit scab. *s*, scab containing crushed resin ducts of skin; *ck*, cork; *pg*, phellogen. $\times 28$.

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SOME OBSERVATIONS UPON THE "RED SPIDER",
TETRANYCHUS TELARIUS L., ON HOPS AND
ITS CONTROL, WITH NOTES ON SOME
PREDATORY INSECTS

BY S. G. JARY, B.A.

(*Entomologist, South-Eastern Agricultural College, Wye, Kent.*)

DURING the summer of 1933, red spider was particularly abundant on hops in Kent, Sussex and many other parts of the country. Attacks of great severity were reported during August and early September, and hops were so badly damaged in some instances that complete loss of crop resulted.

Severe infestations are well known to occur only during periods of drought and especially upon soils which suffer most under those conditions, for, when normal rainfall occurs, it appears to exert such a controlling influence upon the multiplication of the mite that no special measures need be taken to check its increase. In some hop gardens, particularly where the soil is of a retentive nature and does not dry out to a serious extent, the mite is almost unknown, and even in such summers as 1933 and 1934 attacks are negligible.

Most of the information hitherto available concerning control measures is contained in *Cultivation, Diseases and Insect Pests of the Hop Crop*, published by the Ministry of Agriculture and Fisheries (1925). It is stated there that infestation is carried on from year to year by female mites which hibernate in soil around the hills and in crevices of poles or under loose bark. The suggestion is made that flooding the hop gardens during winter, where possible, would destroy the soil-hibernating forms or that the soil might be treated with a crude naphthalene at the rate of 2-3 cwt. per acre. For the treatment of poles, carbolineum (the washes now familiar as tar oil winter washes) could be employed to kill mites hibernating there. For the purpose of dealing with attacks as they arise in the summer, a wash of lime sulphur at 1 per cent. concentration is advised, and the effect of this wash is said to be much improved by the addition of 6 lb. of flour paste to each 100 gallons of wash. Flour paste alone, at the rate of 10 lb. of flour to 100 gallons of water, is stated to do much good by acting as an immobilising agent and causing the mites to

stick to the leaves. Since the washes used for summer application have no effect upon the eggs, they must be repeated at intervals of 10-14 days and two or three applications made.

More recently, Massee (1933) has advised the use of a wash composed of liver of sulphur $\frac{3}{4}$ -1 lb., soft soap 5 lb., water 100 gallons. He indicates that this wash should be used early in the progress of attacks, during July, and spraying repeated in order to deal with mites which subsequently hatch. Liver of sulphur at a strength of 1-2 lb. per 100 gallons of water with soft soap was formerly used to some extent to control hop mould, and this is commented upon by Salmon in *Cultivation, Diseases and Insect Pests of the Hop Crop*: Concentrations up to 4 lb. of liver of sulphur per 100 gallons of water are said to be necessary to kill the mould, but attention is drawn to the danger of using such concentrations in the "pin" and "burr" stages, owing to the possibility of injury.

Experience during the summers of 1933 and 1934 showed that few, if any, of the measures advised for the control of "red spider" were practised by growers. This may perhaps be explained by the fact that attacks are so influenced by weather conditions that a short, wet spell will often eradicate the trouble when a severe attack seemed probable. Thus the treatment of the soil and poles to destroy hibernating mites has not been regarded by hop growers as essential and they have preferred to await the commencement of attacks, relying upon the application of spray fluids in the summer to check them. Liver of sulphur has been used by many growers for this purpose, while others have relied upon frequent and heavy applications of plain water. Some degree of control has been obtained by these methods, but plain water alone is only partially effective during persistent drought, and liver of sulphur suffers from the disadvantage of being a variable and unstable substance which, unless freshly prepared, is liable to cause injury to the hops. No instance is known to the writer of the use of lime sulphur on hops, although this substance is well known to control mites of other species upon a variety of plants. Possibly it was not tried because, in the publication referred to above, no recommendation was made concerning the period during which it could safely be used, and there seems to be no record of experimental work upon the effect of lime sulphur on hops.

The experiments described below were undertaken to investigate further the methods which could be employed against this mite and to study the effect of certain spray fluids upon the hop plant itself.

SOIL TREATMENT WITH NAPHTHALENE.

In November 1933, the effect of a semi-refined form of naphthalene, "Drained Creosote Salts", was tested against the soil-hibernating mites. Duplicate plots received a dressing at the rate of 300 lb. per acre, which was immediately forked into the soil. During the summer of 1934, infestation by *Tetranychus telarius* on the treated plots was similar to that occurring on adjacent untreated ones. It thus appears that, even if such treatment of the soil is destructive to mites hibernating there, serious infestation of hops can arise from other sources.

EXAMINATION OF THE MITE POPULATION OF POLES.

The possibility that serious infestation could arise from mites hibernating in poles was next examined. In early December 1933, a number of poles were taken down from rows of hops which had been attacked in the previous summer. They were all chestnut poles which, as is usual, had developed a single large crack often a quarter of an inch wide at the outside extending down to the centre of the pole. Any other cracks were usually small and shallow. A number of these poles were sawn into 9-in. lengths and each was split down the main crack so that the mite population could be determined. The figures below show the number and distribution of the mites in two typical poles. Each pole was cut into twenty-one sections which were numbered from the top downwards 1-21. In pole 1, section No. 20 extended from about 2 in. below ground-level to 7 in. above, and in pole 2 the section in the same position relative to the ground was No. 19. The numbers in brackets after each section are the numbers of mites found in that section.

Pole 1. 21 (0); 20 (19); 19 (54); 18 (29); 17 (26); 16 (12); 15 and 14 (0); 13 (9); 12, 11, 10, 9 and 8 (0); 7 (3); 6, 5, 4, 3, 2 and 1 (0). Total 152.

Pole 2. 21 (0); 20 (0); 19 (170); 18 (560); 17 (1100); 16 (1410); 15 (410); 14 (470); 13 (430); 12 (320); 11 (2); 10 (70); 9 (40); 8 (110); 7 (0); 6 (10); 5 (0); 4 (30); 3 (10); 2 (0); 1 (10). Total 5152. (Numbers of mites are given to the nearest 10.)

The populations of mites in the two poles represent the extremes of numbers found, and if as many as 5000 mites hibernate in a single pole, this is clearly sufficient to account for an original heavy infestation, even if none takes place from the soil. Unlike the soil-hibernating mites, those in poles are protected from the disturbing influences of moisture and

cultivation which are almost certain to produce a great mortality among the former. The distribution of mites in the poles shows that they do not go far below ground-level; none was found in cracks more than 2 in. below ground-level, and even at this point there were very few. Those which hibernated in the section at soil-level on wood which was affected by creosote treatment of the base of the pole were usually found dead. The great majority of mites occurred from ground-level to about 4 ft. above ground, and thereafter numbers more or less regularly decreased toward the top of the pole. The hibernating colonies consisted mainly of large groups of mites, situated at the very bottom of the crack and often protected by a slight web, but this was not invariably present. An accumulation of dust and debris was often found about half-way down the crack, and this afforded additional protection to the mites, which seemed to remain quite dry unless driving rain penetrated directly into the crack. A few dead mites were invariably found, probably killed by predatory mites which were sometimes present among the colonies, or by the predacious bug, *Anthocoris nemorum*, which was commonly found.

Treatment of poles. In an attempt to discover a suitable method for destroying mites hibernating in poles, experiments were carried out during January 1934 to determine the toxicity of tar- and petroleum-oil emulsions toward the mites. For this purpose, emulsions were made by the two-solution oleic acid method (Martin⁽²⁾) of a Long Ashton type tar oil⁽⁶⁾ and a semi-refined petroleum oil⁽¹⁾. The poles were placed on the ground and the washes applied by means of a pneumatic knapsack machine, using a nozzle giving a heavy spray, which was directed into the crack. Certain lengths of each pole were protected from the wash and afterwards examined as checks.

The emulsions used contained 5 per cent. of the tar and petroleum oils respectively, and they showed a marked difference in toxicity toward the hibernating mites. The tar-oil emulsion was superior to that containing petroleum oil, and this superiority was clearly due to the greater wetting properties of the former. Under the most favourable conditions, with very thorough and careful application, the tar-oil emulsion killed about 90 per cent. of the mites, whereas the petroleum-oil emulsion possessed a maximum toxicity of about 65 per cent. The difference in wetting power of the two emulsions was further shown by dipping completely in the emulsions portions of poles containing colonies of the mites, when similar results were obtained.

SUMMER APPLICATION OF WASHES AGAINST *TETRANYCHUS TELARIUS*
ON HOP LEAVES.(a) *On pot plants.*

A series of trials with various spray fluids was carried out on small hop plants grown in pots, and fourteen separate treatments were tested. These were:

(1) A water-white petroleum oil emulsified with 5 : 7½ : 100 Bordeaux mixture (Pickering (5)): 1 per cent. oil.

(2) As (1), but 2 per cent. oil.

(3) Petroleum oil as in (1) and (2), emulsified by the two-solution oleic acid method (Martin (2)): 2 per cent. oil.

(4) Proprietary Derris preparation at 0.125 per cent., the wash as applied containing 0.0056 per cent. rotenone and 0.036 per cent. sulphonated lorol.

(5) Sulphonated lorol, 0.05 per cent.

(6) Colloidal sulphur 0.3 per cent. + sulphonated lorol 0.05 per cent.

(7) Sodium γ sulphonate (Martin (3)): 0.05 per cent.

(8) Lime sulphur 3.3 per cent. + sulphonated lorol 0.05 per cent.

(9) Lime sulphur 1.6 per cent. + sulphonated lorol 0.05 per cent.

(10) Liver of sulphur 10 oz. per 100 gallons + sulphonated lorol 0.05 per cent.

(11) Liver of sulphur 16 oz. per 100 gallons + sulphonated lorol 0.05 per cent.

(12) Liver of sulphur 24 oz. per 100 gallons + sulphonated lorol 0.05 per cent.

(13) Plain water.

(14) Untreated.

The first application of the washes was given on July 11th and a second on July 20th. A few days after each application, estimation of the effect of the washes was made, from which the following conclusions were drawn.

The emulsions of highly refined petroleum oil at 2 per cent. oil concentration showed a marked toxicity toward the mites. At 1 per cent. the first application did not appear to have much effect, but a second application resulted in complete control. These emulsions caused some foliage injury especially at the higher concentration, and further investigation of their phytocidal effect is necessary before their use on hops can be recommended. The results obtained with the Derris wash showed a marked variation; the first application appeared to be quite

ineffective, whereas the second resulted in almost as complete freedom from mites as the oil emulsions. The two substances, sulphonated lorol and sodium γ sulphonate, which are essentially wetting agents for use with spray fluids, showed no toxicity greater than that of plain water. As sulphonated lorol was used at a uniform concentration as a wetting agent in conjunction with the colloidal sulphur, lime sulphur and liver of sulphur washes, it was necessary to determine whether it possessed any toxicity of its own. Colloidal sulphur at the concentration used was ineffective, and though washes containing liver of sulphur caused some mortality among the mites, they were markedly inferior to lime-sulphur washes. Lime sulphur, at both concentrations, was effective in reducing the numbers of mites to very small proportions, and, since only immature forms were found after the first application it is probable that these had emerged from eggs present at that time. These forms were killed by the second application before they had reached maturity, and thus before further eggs were laid. Plain water alone brought about a slight reduction in the population of mites, but its effect was negligible in comparison with the control exerted by some of the other washes. There was evidence of a certain amount of foliage injury on plants which received the lime-sulphur washes, but, since the plants concerned were growing in very unfavourable conditions, it was not considered that this was serious and, as with the petroleum-oil emulsions, field experiments are necessary to determine how serious such injury may be.

(b) *Laboratory trials.*

The toxicity of the washes used on pot plants was further investigated in the laboratory. For this purpose, the liquids were applied by means of a hand atomiser to leaves of broad beans infested with *T. telarius*, the leaves being kept in covered glass dishes. Under these conditions the resulting mortality of mites was naturally much higher than could be expected in the field, but the results, in the main, confirmed those obtained on the pot plants. The Derris wash showed a consistently high toxicity under laboratory conditions and no explanation can be offered for the failure of this same wash to exert a control at the first application as recorded above in the pot experiment. The erratic behaviour of Derris washes against this mite has, however, often been brought to the writer's notice by growers during the past two years, and a preliminary small-scale laboratory experiment was carried out to investigate the matter further, using the same technique as that formerly employed. Although the differences shown were small, in-

dications were obtained that the toxicity of a Derris wash to the mite may be greater some hours after its preparation than when freshly prepared.

(c) *Field experiments.*

Through the kindness of Mr S. A. Robson, Chartham, Kent, it was found possible to try out a number of washes in the field on badly infested hops. A row of hills of the variety Cobbs was selected, where a serious attack was experienced in 1933. Eight treatments (as given below) were tested out in duplicate, the unit for each treatment being three consecutive hills. All washes were applied by means of a single fruit tree spray lance operated by a headland pump at about 100 lb. pressure. The washes were directed upward so as to hit the undersides of the leaves. The hops were several years old and in full cropping, requiring an average of about two-thirds of a gallon of wash per hill. Two applications were given, the first on June 27th and the second on July 6th. Except for a heavy shower which fell about an hour after the first application, weather was fine and warm over the period. After the second application, all wires and strings between each block of three hills were ringed with tree banding grease in order to prevent possible migration of mites. The first estimation of results was made on July 21st, observations being continued until August 29th; about thirty leaves, mainly the lower and larger ones, were examined on the hills under each treatment. The lime-sulphur treatments were specially thoroughly examined and the whole bine inspected with the aid of a ladder.

No. of hill	Treatment
1-3, 25-27	(1) Sulphonated lorol, 8 oz. per 100 gallons
4-6, 28-30	(2) Sodium γ sulphonate, 8 oz. per 100 gallons
7-9, 31-33	(3) Colloidal sulphur, 3 lb. + sulphonated lorol, 8 oz. per 100 gallons
10-12, 34-36	(4) Lime sulphur, $1\frac{3}{4}$ gallons + sulphonated lorol, 8 oz. in 100 gallons of wash
13-15, 37-39	(5) Liver of sulphur, 10 oz. + sulphonated lorol, 8 oz. per 100 gallons
16-18, 40-42	(6) Liver of sulphur, 16 oz. + sulphonated lorol, 8 oz. per 100 gallons
19-21, 43-45	(7) Plain water
22-24, 46-48	(8) Untreated

Treatment	Observations
(1) Sulphonated lorol	Mites common but not abundant; many eggs.
(2) Sodium γ sulphonate	Mites common but not abundant; many eggs.
(3) Colloidal sulphur	Mites common and some eggs; not quite so abundant as on (1) and (2).
(4) Lime sulphur	A single adult mite and a few eggs, very high up on one bine, otherwise no live mites or eggs. Some lower leaves scorched and falling.
(5) Liver of sulphur (10 oz.)	Mites and eggs common. Very slight leaf injury.
(6) Liver of sulphur (16 oz.)	Mites rather less numerous than on (5) but eggs common. Very slight leaf injury.
(7) Water	Mites and eggs common but very irregular in distribution.
(8) Untreated	Mites and eggs common but irregular in distribution.

The only satisfactory control of mites was that given by the lime-sulphur treatment, and the freedom of these blocks was most marked. Some scorching of leaves and lower lateral growth was caused by this wash, but such injury was confined to the lower parts of the bine, the younger leaves toward the top, from about 3 ft. above the breast wire, being quite normal. It is possible that this effect may have been due to the interaction of lime sulphur with Bordeaux mixture, applications of the latter being given to all hills under this experiment as well as those in the remainder of the garden. The second application of lime sulphur was actually given immediately after an application of Bordeaux mixture and before the latter was dry. A black deposit, presumably due to copper-sulphur combination, resulted. Since lime sulphur was the only wash tested which gave promising results, it was decided to investigate further the effect of this substance upon hops, and in particular the stage of growth of the plant in relation to its susceptibility to injury.

THE EFFECT OF LIME SULPHUR ON HOPS; PHYTOCIDAL ACTION.

The applications of lime sulphur in the above experiments were made at a comparatively early stage in the progress of attacks and, although it is probably wise to put control measures into operation as soon as possible in some seasons, it is well known that the onset of moist weather conditions will quickly eradicate severe infestations. On the whole, growers are most concerned about infestations present when the "burr" stage of the inflorescence is reached, and the experiments described below were undertaken primarily to discover whether lime sulphur would cause injury at such a period. Mr S. A. Robson was again good enough to provide facilities for carrying out a field scale trial. Two-year-old hops of the variety Fuggles, making very good growth, were found to be heavily infested with mites, and these plants had received no previous applications of any insecticide or fungicide. At the date of application of the wash, they were in "burr", all stages from very young "burr" to full "brush" being present, but no cones had formed. Five concentrations of lime sulphur were used, containing respectively $1\frac{2}{3}$, $1\frac{1}{4}$, 1, $\frac{5}{8}$ and $\frac{3}{4}$ gallons of lime sulphur per 100 gallons of wash. The lime sulphur used was a well-known proprietary brand and no spreader was added. The washes were applied with the same headland machine as used in other experiments, and a single lance was employed. A pressure of about 120 lb. was maintained and the whole of the bine was very thoroughly wetted and made to drip, the quantity given to each plant being much greater than that applied by an ordinary hop-washer. Each of the five

concentrations of lime sulphur was applied to eleven consecutive hills on July 31st. An examination made on August 10th revealed the fact that no injury whatever had been caused either to the foliage or the "burr". The two weakest concentrations of wash had given an indifferent control of the mites, which were still rather numerous on the hills thus treated, but the three strongest concentrations had brought about a marked reduction in their numbers. No adult mites were present, but some numbers of immature forms could be found, which had, in all probability, hatched from eggs present at the time of washing, and it appeared that all live mites then present had been destroyed. By this date, small cones had formed and these were normally seeded, though they might have been fertilised before the application of the wash. A second examination, made on August 16th, confirmed the previous observations, normal seeded cones being produced everywhere with no injury whatever, and, except on the plants which had received the two weakest concentrations, a good control of the mites had been obtained from the one application of wash. At about this period, showery weather commenced and no appreciable increase in infestation took place on any hops in the district, less injury being caused, on the whole, in east Kent, than in the previous year.

A small experiment of the same nature was carried out at Wye on two other varieties of hops. The same five concentrations of lime sulphur were used, but sulphonated lorol was added as a spreader to each one at the rate of 8 oz. sulphonated lorol per 100 gallons of wash. The washes were applied to individual sprays of hops at the various stages of development shown below on August 1st:

- (a) Variety Early Birds: Small cones.
- (b) Variety Early Birds: "Burr" pollinated with tips just beginning to shrivel.
- (c) Variety Cobbs: Young "burr", partly pollinated.

Six sprays of each were treated and labelled, the whole of the remainder of the plant concerned being untreated. Examination on August 14th showed that wherever cones in any stage of development were washed, brown discoloration resulted and the injury was apparent even with the weakest concentration of lime sulphur. Where "burr" alone was present, no injury whatever was caused, thus confirming the results obtained at Chartham on the variety Fuggles.

NOTES ON SOME INSECTS PREDATORY UPON *TETRANYCHUS TELARIUS*
ON HOPS.

During the experiments above described, a number of insects were observed to be predatory upon the Hop Red Spider, two of which do not appear to have been recorded in this country before as predators of this mite.

(1) *Anthocoris nemorum* L. This very common bug was usually to be found among mite colonies and its nymphal forms especially were active predators. The younger nymphs moved freely about beneath the web made by the mites and seemed to feed mainly by puncturing and sucking eggs. Older nymphs readily attacked living mites and were not observed to feed to any extent upon the eggs. Adults of *A. nemorum* were often found hibernating in cracks of hop poles among colonies of the mites.

(2) *Scymnus punctillum* Weise (*minimus* Rossi). Fowler records this beetle as having been taken on hops and by beating dead hedges and sweeping herbage. It seems to be confined largely to the south-eastern counties, but *Scymnus* larvae have been reported as predatory upon aphides in France, and recently in New Zealand a related species was noted as a predator of the Fruit Tree Red Mite. In this country its activities appear to have been overlooked, but in 1934 it was found to be relatively common in many hop gardens, though its small size, 1-1½ mm., makes it very inconspicuous. Eggs were found, laid singly on the undersides of hop leaves among colonies of *Tetranychus telarius*, and the larvae actively devoured both mites and their eggs. The larvae pupated among the webs formed by the mites and the life cycle was completed in 5-6 weeks.

(3) *Feltiella tetranychii* Rubsaamen. Hop leaves heavily infested with *Tetranychus telarius*, received from the neighbourhood of Wadhurst, Sussex, were found to have many Cecidomyid larvae on them. These larvae fed upon the mites and adult flies were eventually bred from them which Dr H. F. Barnes identified. This is apparently the first authentic record of the species in this country.

SUMMARY.

1. Treatment of the soil around hops in November, with crude naphthalene at the rate of 300 lb. per acre, did not prevent infestation by Hop Red Spider in the following summer.

2. Examination of hop poles showed that large numbers of mites may hibernate in deep cracks in the poles.

3. An emulsion containing 5 per cent. of a high-boiling neutral tar oil, sprayed with force into the cracks of poles, killed the great majority of mites. It was more effective in this respect than an emulsion containing 5 per cent. of a semi-refined petroleum oil.

4. Of a number of washes applied in the summer to infested hops, emulsions of a highly refined (water-white) petroleum oil at 1 and 2 per cent. oil concentrations, were effective in killing the mites. Lime sulphur at concentrations of 1 in 30 and 1 in 60 also gave a complete control. A Derris wash of 0.0056 per cent. rotenone content appeared to give a complete control at the second application whereas it failed to do so at the first. There is evidence that the toxicity of a Derris wash of the type used increases up to a point with time after water is added to the powder. Liver of sulphur and colloidal sulphur at the concentrations used showed no marked toxicity and the two spreaders used were also non-toxic.

5. The petroleum-oil emulsions and lime sulphur caused some injury to foliage when applied in late June and the first three weeks in July.

6. Lime sulphur caused no injury to foliage or "burr" on the varieties Fuggles, Cobbs and Early Birds, when applied during late July and early August, at concentrations ranging from 1 in 60 to 1 in 150. Concentrations smaller than 1 in 100 were not markedly toxic to the mites, but at 1 in 60 and 1 in 80 lime-sulphur wash was completely effective in killing all forms other than eggs.

7. Notes are added on three insect predators of the Hop Red Spider.

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STUDIES ON APHIDES INFESTING THE POTATO CROP

IV. NOTES ON THE MIGRATION AND CONDITION OF ALATE *MYZUS PERSICAE* SULZ.

BY W. MALDWYN DAVIES, B.Sc., PH.D.

AND T. WHITEHEAD, M.Sc., PH.D.

(*University College of North Wales, Bangor.*)

THE rôle of *Myzus persicae* Sulz. as a vector of virus diseases is generally recognised, but little is known of the biology of this species in relation to the dissemination of disease. Experimental data are being obtained in the laboratory (Davies⁽⁴⁾) on the effect of the different meteorological factors upon migration and reproduction, and it is essential that these data should be supported by field observations. Further, no information exists on the proportion of migrating alatae that are infected with virus diseases.

In order to understand fully the part played by *M. persicae* in dissemination it is necessary to follow its seasonal development. Previous studies (Davies⁽³⁾) have shown that, in addition to over-wintering in the egg stage on nectarine and peaches, this aphid more generally hibernates as apterae on various cruciferous plants. Apterous generations are produced, very slowly but continuously, throughout the winter.¹ On the approach of warm weather in spring, the apterous progeny increase and reproduce rapidly. A few winged females may appear in the colonies as early as March or April but, often, their wings are malformed by the excessive damp conditions in these months. Conditions at this period do not favour migration, and the little that takes place is, inevitably, local. Some of the early winged forms are able to migrate in May, as is evident by the fact that seedling charlock, shepherd's purse and other cruciferous weeds are infested in varying degree. The first potatoes appear above ground, in the field, about mid-May, and they are not common until late May and early June. How do these potatoes become infested with *M. persicae*? Infestation with apterae from adjacent

¹ Experiments have shown that individuals crawling from potatoes in the autumn cannot themselves survive until the spring and thus provide the source of virus disease the following year.

cruciferous weeds can occur, but only to a slight extent, since the potato drills will have been newly formed and cultural methods adopted at this period destroy most of the weeds. Infestation of the tubers before planting cannot be overlooked as an initial source of the field population (Davies(2)), but again it is likely to be of rare occurrence. The more general form of initial infestation of the potato crop in the field is by migrating alatae. This is borne out by field observations since 1928, inspection having shown that for some time after the appearance of the leaves above ground the plants remain comparatively free from aphides. Then, suddenly, following records of quantities of migrating alatae, the aphis population rapidly increases. It is important that the factors influencing this migration and its intensity should be studied in detail.

In 1934, a season of heavy infestation of aphides, an opportunity was provided for studying this main migration in the field at a centre at Holywell, Flintshire (200 ft. above sea-level, fairly open), known for its previous heavy infestations and also for the fact that in this locality stocks rapidly deteriorate owing to the introduction and spread of virus diseases (Whitehead, Currie and Davies(1)). Observations were commenced on May 7th when an adhesive muslin trap—one yard square and set to face the wind—was erected in a potato field. Examination of the trap on May 14th and May 28th showed that migration of winged aphides was negligible. The planting on the experimental field was delayed until May 28th, but inspection of the few early plants above ground in adjacent fields showed that migration had not commenced. The potatoes appeared above ground during the last few days of June; this was late but proved an advantage in the observations on migration. Daily observations were made from June 26th, but alate *M. persicae* did not arrive in any quantity until July 6th when fifty-five alatae were collected from the potatoes in approximately seven hours. The numbers increased rapidly on July 9th, 10th and 11th, so that on July 12th, 235 alatae were collected and the index figure was twenty-three alate *M. persicae* per 100 leaves taken at random, with practically 100 per cent. of the plants infested. It was very evident that meteorological conditions were ideal for migration during the period July 6th–11th. At this centre it was not possible to make meteorological recordings, but acknowledgment is due to the Air Ministry for the loan of the Meteorological Register made at Sealand, Flintshire, also in the Dee Estuary some nine miles from this centre. These records show that the period July 5th–11th differed markedly from either the preceding or the subsequent week. During this period the mean maximum temperature

during the daytime was 86.7° F., whereas in the previous week it had been 67° F. and in the subsequent week it was 72° F. Similarly the relative humidity taken at 1 p.m. (1300 G.M.T.) during July 5th–11th gave an average daily value of 34.8 per cent., while in the previous week it had been 68.5 and in the subsequent week it was 69.5 per cent. The wind records for the period also contrasted, for from June 28th to July 4th the wind was north-west or north-west-north and at 1 p.m. averaged 16 m.p.h. On July 5th the wind changed to south-east and remained in that quarter until the 11th, averaging at the same period 6 m.p.h. On July 12th the wind again changed to north-west and varied from north-west to west during the following week, while the velocity averaged 15.7 m.p.h.

A further instance of this correlation of migration with weather conditions was shown by the decline of alatae after July 12th followed by a sudden increase on the 21st; on this date the wind again changed to south-south-east, the maximum temperature rose to 81° F., and the humidity fell to 44 per cent. It is concluded that the heavy migration of alate *M. persicae* during July 5th–11th, undoubtedly, was due to the combination of high temperatures, low humidities and slight velocity of the wind. Observations of this character will be continued in detail in subsequent seasons in an endeavour to ascertain more precisely the interrelationship of the several meteorological factors affecting migration, but it cannot be expected that such contrasting meteorological conditions will often coincide with the period of field observations.

The sudden appearance of such large quantities of alate *M. persicae* on the potato plants prompted a search for their host plants. The more advanced potato crops in the district were examined but the nymphs on the potato leaves showed no signs of producing wing buds and the alatae present had not the delicate characteristics of newly developed adults. Ultimately it was discovered that charlock, shepherd's purse and other small weeds were heavily infested with *M. persicae*. The very dry conditions in June and early July had caused these small weeds to wilt and even die with the result that the apterous colonies gave way to alate forms and practically all the nymphs examined in such sites were developing wings, while newly formed alatae were abundant. The dry conditions, therefore, not only encourage flight (Davies(4)) but also increase the proportion of alatae through the wilting of some of the weed host plants.

Since migration was greatest when the wind was from an easterly direction an attempt was made to ascertain whether alatae migrate across

the Dee Estuary, which lies to the east of the experimental field. Through the kindness of G. V. Waine, Esq., adhesive muslin traps and nets were erected on the terrace of the Point of Air Lighthouse, situated at the mouth of the estuary and some 2 miles from the experimental field. During the periods May 28th—June 6th—June 20th—July 12th—July 20th no alatae of any kind were caught. From July 20th to August 3rd twelve alatae (no *M. persicae*) were observed on the adhesive muslin, only four of these were on the east side. In addition to the traps and nets, which failed to catch any aphides, sprouting healthy half-tubers were exposed with a view to attracting alate *M. persicae* and determining their virus condition. No aphides were found on the sprouts from May 28th to August 3rd. The very small number of alatae obtained at the lighthouse, while indicating that near the sea-level migration was negligible, possibly owing to a high humidity, cannot be regarded as satisfactory evidence of the absence of migration across the Dee Estuary, for in view of other data obtained during the season it will be necessary to examine the fauna at higher levels of the atmosphere before a final opinion can be given.

A comparative study was also made of the infestation of alate *M. persicae* at the centre already referred to, with that at a centre at Aberdaron, South Caernarvon—one of the most successful seed-potato producing centres, where the stocks have remained practically free from virus infection for over seven years. It had been shown (Davies(3)) that the population of apterous *M. persicae* on the potato crop at Aberdaron is considerably below that at Holywell. The observations in 1934 on alatae at the latter centre were, therefore, compared with the infestation on the plants at Aberdaron. The comparison was striking, for at all periods alate *M. persicae* were rare on potatoes at Aberdaron. For instance, on July 13th, the day following the maximum daily collections at Holywell, the index figure at Aberdaron not only gave 0 per 100 leaves, but careful search of the plants for six hours failed to yield a single alate specimen. This marked difference in the number of alate *M. persicae* at the two centres is supported by the number of migrating aphides (of various species) taken on adhesive traps, consisting of three separate strips of muslin, 4 by 36 in., smeared with an adhesive mixture. During the period of maximum migration in 1933, 738 migrating aphides were taken during ten days at Holywell, whereas at Aberdaron during the same period and on a similar area only twenty alate aphides were caught. In the previous laboratory experiments (Davies(4)) it was suggested that the high humidities, including sea mists, common in the

Aberdaron district, might be largely responsible for this contrasting difference in the intensity of the population of alate aphides at the two centres. While, in the meteorological records, the factor of humidity stands out in contrast at these two centres and supports this conclusion, the other separate meteorological factors are being investigated in relation to the migration of alatae.

VIRUS CONDITION OF MIGRATING ALATE *MYZUS PERSICAE*.

It was important in view of the rôle of *M. persicae* in the dissemination of virus diseases to ascertain the degree of infection of migrating alatae in the *main* migration. The centre at Holywell, in a district where increase of virus infection among the potato stocks was known to be rapid and where heavily infected crops were present on adjacent farms, proved admirable for these experiments. Attempts were made to trap migrating alatae in flight and various types of nets were devised, but the quantities of the species *M. persicae* caught were too small for the present purpose. It was decided, therefore, when the main migration commenced, to take alatae after they had alighted on a healthy crop of potatoes. The stocks for this purpose were secured from two sources: 5 cwt. of Welsh certified seed grown near Portmadoc, South Caernarvon, where the incidence of virus disease has not exceeded 0.23 per cent. in the last seven years; and an equal quantity of Scotch Stock seed obtained from Banffshire. No other seed potatoes were planted on the farm, so that the possibility of the alate aphides having picked up the virus diseases within the experimental field was negligible.

Alate aphides were collected on the leaves and taken to the laboratory at Bangor where they were identified and placed on sprouted healthy half-tubers; the corresponding halves were planted as controls. At first, five alate *M. persicae* were placed on each half-tuber, but later, when the numbers increased, twenty individuals were transferred in each case. Each infested half-tuber was then isolated and examined at intervals when feeding of the aphides was noted. Infestation was allowed to continue for 14 days, when the alate forms were removed and the half-tubers were dipped in a nicotine solution to kill off the nymphs; they were then planted and ultimately observations were made on the virus condition of the plants, which were grown in an insect-free glasshouse. The results are given in Table I. The observations show that 1178 alate *M. persicae* were collected from potato foliage during the period June 15th to July 26th; 140 of these (Exps. 1-7, 13-16, 18, 22, 23-24) were taken from another centre where the stocks were not free

from virus infection. During the last days of July a few insects bearing wing buds were observed on the potatoes at the experimental centre, and collecting was then discontinued in order to exclude alatae that had been bred up on this particular crop and had not migrated. The population of alate *M. persicae* rapidly declined towards the end of July and in early August; this has been generally the case during the last seven years. It

Table I.
*Experiments on the virus condition of migrating
alate Myzus persicae.*

Exp.	Date of collection	No. of <i>Myzus persicae</i>	Plant from infested half-tuber	Plant from control half-tuber	Exp.	Date of collection	No. of <i>Myzus persicae</i>	Plant from infested half-tuber	Plant from control half-tuber
1	June 15*	5	Healthy	Healthy	42	July 10	20	Healthy	Healthy
2	" 15*	5	"	"	43	" 10	20	"	"
3	" 15*	4	"	"	44	" 10	20	"	"
4	" 15*	5	"	"	45	" 11	20	"	"
5	" 23*	5	"	"	46	" 11	20	"	"
6	" 23*	5	"	"	47	" 11	20	"	"
7	" 23*	5	"	"	48	" 11	20	"	"
8	" 26	5	"	"	49	" 11	20	"	"
9	" 27	5	"	"	50	" 11	20	"	"
10	" 27	5	"	"	51	" 11	34	"	"
11	" 27	5	"	"	52	" 12	20	"	"
12	" 27	5	"	"	53	" 12	20	Leaf-roll	"
13	" 28*	5	"	"	54	" 12	20	"	"
14	" 28*	5	"	"	55	" 12	20	Healthy	"
15	" 28*	5	"	"	56	" 12	50	Mosaic	"
16	" 29*	20	"	"	57	" 12	50	Healthy	"
17	" 29	20	"	"	58	" 12	50	"	"
18	July 4*	2	"	"	59	" 17	20	"	"
19	" 5	5	Leaf-roll	"	60	" 17	20	"	"
20	" 5	5	Healthy	"	61	" 17	20	"	"
21	" 5	5	Leaf-roll	"	62	" 17	14	"	"
22	" 5*	5	Healthy	"	63	" 18	20	"	"
23	" 5*	5	"	"	64	" 18	20	"	"
24	" 5*	3	"	"	65	" 18	11	"	"
25	" 6	5	Mosaic	Mosaic	66	" 18	20	"	"
26	" 6	5	Healthy	Healthy	67	" 18	22	"	"
27	" 6	5	Mosaic	Mosaic	68	" 21	20	"	"
28	" 6	5	Failed	"	69	" 21	20	"	"
29	" 6	5	Healthy	Failed	70	" 21	20	"	"
30	" 6	5	"	Healthy	71	" 21	20	"	"
31	" 6	5	"	"	72	" 21	20	Leaf-roll	Leaf-roll
32	" 6	5	"	"	73	" 21	20	Mosaic	Failed
33	" 6	5	Mosaic	"	74	" 21	20	Healthy	Healthy
34	" 6	5	Healthy	"	75	" 21	9	"	"
35	" 6	5	"	"	76	" 24	20	"	"
36	" 9	20	"	"	77	" 24	20	"	"
37	" 9	20	"	"	78	" 24	20	"	Failed
38	" 9	20	"	"	79	" 24	10	"	Healthy
39	" 10	20	"	"	80	" 26	20	"	Failed
40	" 10	20	"	"	81	" 26	9	"	Healthy
41	" 10	20	"	"	Total 1178				

* Not from experimental field.

will be seen from Table I that only in four instances was there transmission of leaf-roll; two of these (Exps. 19 and 21) involved five alatae each and the other two (Exps. 53 and 54) each involved twenty alatae. From the fact that the other seventy-five experiments (excluding 28 and 72) involving 1103 alatae did not include vectors it seems likely that only a small proportion, possibly only one individual in each of the four experiments, were infected. Exps. 25 and 27 were inconclusive since both controls and infested plant showed slight mosaic, but so far as leaf-roll is concerned these also can be regarded as negative. Exp. 28 was inconclusive owing to the failure of the plant, and Exp. 72 was discarded since the control was infected with leaf-roll. So far as mosaic is concerned Exps. 33 and 56 alone showed evidence of transmission of a very mild form.

The results, therefore, indicate that even in a district where spread of virus diseases is rapid, an extremely small proportion of migrating alate *M. persicae* in the field is infected with virus diseases. This small infection, however, must suffice to introduce disease into healthy stocks, and subsequent spread within the crop will be through infected apterae. This conclusion is supported by the fact that in 1927-30, when observations were made on the adjacent farm (see (1) Farm J), the introduction of virus disease in the first year was slight—0.48 per cent. of the plants infected, and in the second year it had only increased to 1.63 per cent., but in the third year there was a rapid rise to 12.98 per cent. Had a large number of the migrating alatae been infected, a rapid increase even in the first year would have been expected.

SUMMARY.

1. Field observations show that migrating alatae are the *main* source of the initial infestation of *Myzus persicae* Sulz. on the potato crop. They arrive in quantity, during June and July, from various cruciferous plants on which they have hibernated, or to which they migrated in early spring.

2. In 1934 the main migration at a selected centre occurred during a week which contrasted with other periods, since the wind, which was slight, changed from the north-west to the south-east, the temperature rose from about 67° F to 80° F. and the relative humidity dropped from 68 to about 30 per cent.

3. The hot, dry conditions not only facilitated flight but also greatly increased the proportion of alatae by causing the cruciferous weeds to wilt and die, when alate forms were produced in abundance.

4. The proportion of *alate M. persicae* was found to be much higher in a district where virus infection of the stocks had been rapid compared with a district where such was negligible.

5. The proportion of migrating *alate M. persicae* infected with virus diseases, in a district where the spread is rapid, proved to be particularly small. In eighty-one experiments involving 1178 *alatae* only four instances included vectors; possibly only a single vector in each of the four experiments.

6. It is concluded that the introduction of virus diseases into a healthy stock by migrating *alatae* is slight, but this small amount is subsequently spread by *apterous* forms moving within the crop.

The writers wish to acknowledge, gratefully, the valuable assistance of Mr Morgan Wynn Griffith who was largely responsible for the collection of the *alatae* and who also assisted in obtaining data on migration.

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ON THE BIOLOGY OF *ARAECERUS FASCICULATUS* DE GEER (COL., ANTHRIBIDAE), WITH
SPECIAL REFERENCE TO THE EFFECTS
OF VARIATIONS IN THE NATURE
AND WATER CONTENT OF THE
FOOD

BY M. TAHER EL SAYED, A.R.C.S., PH.D., F.R.E.S.

(With 4 Text-figures.)

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INTRODUCTION.

ARAECERUS FASCICULATUS DE GEER is an Anthribid beetle of considerable economic importance as a pest of stored products. It is supposed to be indigenous in India, East Indies and the Malay States, but its occurrence is now more or less cosmopolitan, though it is more common in tropical and subtropical countries. It was first described by De Geer in 1775(9). Lucas in 1861(15) recorded it boring into the branches of the Chinese ginger in France. Reh(21), discussing its economic importance, mentions its occurrence on hard seeds, thick-skinned bulbs, palm seeds, nuts of *Areca*, roots of ginger, coffee beans, nutmeg, mace, *Tamariscus*, Euphorbiaceae, cotton seeds, cacao beans, dried apples, peaches and oranges. According to him, its geographical distribution includes India (where it is indigenous), Europe, U.S.A. (Louisiana and Florida), Central America, French Guiana, Bermuda, Brazil, St Helena, Persia, Ceylon, Java, China, Japan, Sandwich Isles and Philippine Isles. Reh thinks that *Araecerus fasciculatus* has only one generation a year and, in agreement with Lucas, mentions about two weeks for the pupal period.

Tucker(25, 26) records its occurrence in cornstalks in fields adjacent to cotton in Louisiana, and points out that it is found in association with cotton-boll weevils in the cornstalks. He states that the insect is sometimes a scavenger, feeding on the decayed cotton bolls.

Again, Van der Goot (28) records it as a field pest on *Tephrosia candida* in Java and calls it "the Tephrosia beetle". He points out that the Tephrosia beetle (which was identified by European entomologists as *Araecerus fasciculatus* De Geer) does not attack stored coffee or cacao in Java, although the plants are attacked by *A. fasciculatus* in the field. It is also interesting to note that, according to Van der Goot, *Tephrosia vogeli* is immune from the attacks of the Tephrosia beetle even when grown among seriously infested trees of *T. candida*. The beetle thus appears to have developed a very marked food-preference instinct, considering that the infestation of *T. candida* is caused by beetles flying from wild Leguminosae, especially *Crotalaria striata*. On the other hand, *Araecerus fasciculatus* as a stored product insect is polyphagous to a great extent, feeding on all sorts of seeds and grains, especially if they are not dry. It can also live to a certain extent on flour, thrives on biscuits and even ordinary bread, preferring the crumb to the crust. There is, therefore, some uncertainty whether the "Tephrosia beetle" is really the same species as the true *A. fasciculatus* De Geer.

Rutgers (22) also seems to doubt the identity of the Tephrosia beetle, stating that *A. fasciculatus* severely attacked a sample of Liberian coffee, but that either this beetle or a closely related one attacks the seeds of *Crotalaria* and *Tephrosia*.

Cotton (6) has briefly described the external appearance of the four stages of the insect, and Autuori (1) published a short external description of all the stages with a reference to the biology. His work was concerned with *Araecerus* as a pest on Brazilian coffee.

Lefroy (14) states that *A. fasciculatus* or "a very closely allied species breeds freely in old, dried cotton seeds that remain on the plant after picking". If Lefroy's illustrations are correct, the insect concerned cannot, however, be *A. fasciculatus*.

Ogilvie (18) records the species on stored maize in Bermuda, where it is also found associated with the "black tip" disease of banana which causes a black discoloration of the skin of the fruit, and this is subsequently attacked by the larva of *A. fasciculatus*. It is recorded damaging the boll and seeds of the cotton plant in Africa (Zacher (29)), attacking cacao in the Gold Coast in the drying stages and then in the stores (Patterson (20)), on cacao pods in Nigeria (Lamborn (13)), in coffee berries in Dutch East Indies (Friederichs (10)), where it also attacks Brazil nuts (Gater (12)), on areca catecha—Papilionaceae in India (Beeson (2)), on monkey-pod (*Samanea saman*) in Hawaii (Bridwell (4)) and as a carrier of the fungus *Diplodia* in the Philippines where it attacks a number of

plants in storage, including roots, seeds and fruits (Sarmiento⁽²³⁾). Tucker^(25, 26), Van der Goot⁽²⁷⁾, Crawford⁽⁷⁾ and Bridwell⁽³⁾ have referred to parasites of the beetle; one of these is a mite belonging to the genus *Pediculoides* and most of the rest are Hymenopterous, two being Bracónids.

Araecerus fasciculatus as a British insect goes back to 1831 when it was recorded by Stephens⁽²⁴⁾ under the name *Phloeobius griseus* Fab. In 1908 Day⁽⁸⁾ recorded it in a biscuit factory in Carlisle, and it has been reported by Munro and Thompson⁽¹⁷⁾ at London docks on consignments of nutmeg from Grenada and East Indies; also in West African and Panama cacao, and occasionally in cacao from Venezuela, Ecuador, and Ceylon. They remark that the insect "although abundant in Grenada nutmegs, occurred in only one of about a dozen consignments of Grenada cacao examined". There are several more references indicating the geographical distribution of the insect and the infestation of a great variety of foods.

THE LIFE CYCLE IN RELATION TO THE ENVIRONMENT.

(1) *Technique.*

Before attempting to study the life history of the insect in different humidities, it was found necessary to determine the absorption of water for the three foods used in the experiments, namely, maize, cacao, and nutmeg. Sound samples of these were heated in a steam drying oven for several hours till the difference in weight between successive weighings was negligible. This method was not, however, successful with nutmeg, as these crack when heated and a considerable amount of oils and fats come out. The equilibrium points of unheated nutmeg in different humidities were therefore determined instead. Sulphuric acid solutions to give 10, 30, 50, 60, 70, 80 and 90 per cent. relative humidities¹ were prepared 5 days before they were required, and distilled water was used for 100 per cent. R.H. Weighed amounts of the dry foods were placed in desiccators containing these solutions and were reweighed every 3 days for the first 18 days and then every 6 days. The specific gravity of the solutions was also measured each time. A slight change was noticed at the time of the first three weighings; after that the specific gravities were constant. With nutmeg, only humidities from 60 per cent. upwards

¹ The concentrations of sulphuric acid corresponding to the various humidities were obtained from tables brought by Mr G. V. B. Herford from the University of Minnesota. They were prepared by Mr Gray, but have not been published.

were used. The figures for 90 and 100 per cent. R.H. in cacao, maize and nutmeg are not reliable in the later stages owing to mould. See Table I.

Table I.

Percentage moisture content of foods at 27° C. at different relative humidities and times taken to reach an equilibrium.

Food	50 % R.H.		60 % R.H.		70 % R.H.		80 % R.H.		90 % R.H.		100 % R.H.	
	% water	Days	% water	Days	% water	Days	% water	Days	% water	Days	% water	Days
Maize	6.7	39	8.4	42	10.1	47	13.5	56	16.9	Mouldy after 48 days	21.2	Mouldy after 15 days
Cacao	2.9	24	4	36	5.1	42	7.9	50	10.5	Mouldy after 36 days	13.3	Mouldy after 15 days
Nutmeg	It is extremely difficult to determine the water content of nutmeg. The unheated sample was nearly in equilibrium with 70 % R.H. It took 30, 9, and 64 days to get into equilibrium with 60, 70 and 80 % R.H. respectively. The nutmeg became mouldy in 90 % R.H. after 54 days and in 100 % after 36 days											

For the biological work it was thought better to substitute potassium hydroxide solutions for sulphuric acid solutions for the following reasons:

(1) Carbon dioxide produced by the seeds or by the insects when accumulated in the desiccator may (see Buxton(5)) cause the spiracles to open widely and increase the loss of water which diffuses through the tracheae.

(2) Sulphuric acid might have a toxic effect.

(3) Potassium hydroxide solutions absorb carbon dioxide and potassium carbonate is formed. According to Paranjpe(19), this has no great effect on the vapour pressure of the atmosphere of the desiccator unless a considerable proportion of carbonate is formed. To avoid this, solutions were not used for more than 45 days. Humidities were measured by paper hygrometers and these were compared every week with wet- and dry-bulb hygrometer. Potassium hydroxide solutions are not in equilibrium with the atmosphere of the desiccator in the first 4 or 5 days, and it was found advisable to prepare them one week before they were required.

The data used in preparing the different humidities were obtained from curves plotted from Paranjpe's data of the vapour pressure of water over aqueous solutions of potash at certain temperatures. The relative humidity of the air over these solutions was calculated irrespective of temperature. These curves were very similar to those of Buxton(5), but were drawn on a larger scale.

The foods used for the life-history work were unheated. The maize and cacao were allowed the necessary periods to come into equilibrium with the respective humidities, the water content of the samples having been previously determined. Nutmeg was placed in desiccators with an atmosphere of 60, 70, 80, 90 and 100 per cent. R.H. and a sample taken and weighed until the change in weight was negligible.

Unless otherwise stated, all determinations of biological constants were made at a constant temperature of 27° C. and the insects were kept with maize in equilibrium with 90 per cent. R.H.

(2) *Emergence of the sexes on maize and nutmeg.*

The experiments dealing with the minimum length of the life cycle (see p. 570) were prolonged to determine the sex ratio of the adults. Emerging adults were looked for daily. In nearly all cases the females

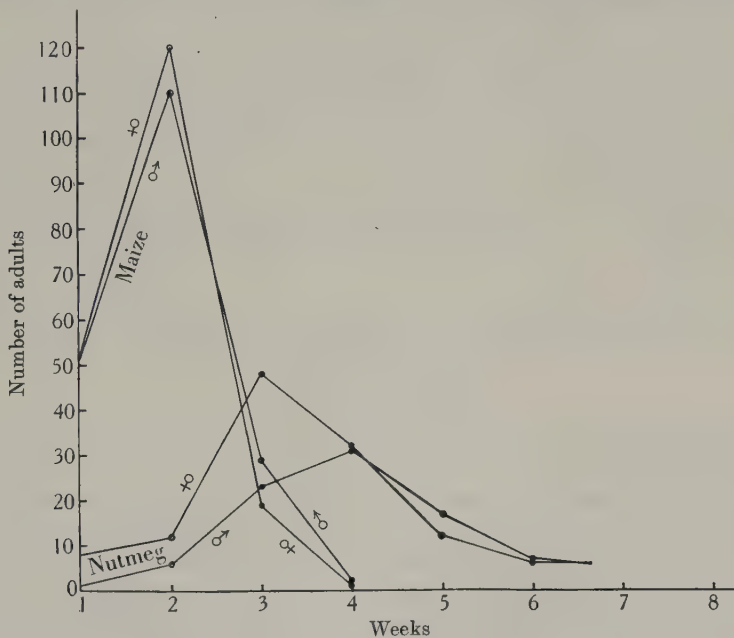


Fig. 1. Emergences of the sexes on maize and nutmeg at 100 per cent. R.H. and 27° C.

were more numerous than the males. Only when bred in maize in equilibrium with 90 and 100 per cent. R.H. was the sex ratio nearly equal. Although the number of females is more than that of the males in lower humidities, the total number of adults in these experiments was really

too small to make a reliable deduction. On the whole, the percentage of the males is from 42 to 46 per cent. The sex ratio calculated from 460 adults collected from consignments of nutmeg in the London docks was forty-three males to fifty-seven females.

From twenty-six females and twenty males which were allowed to lay eggs for 5 days on maize in equilibrium with 100 per cent. R.H. 192 males and 191 females emerged; from the same number of females which laid their eggs on nutmeg in the same relative humidity and for the same period 98 males and 130 females emerged. The females used in both these instances were about 3 weeks old.

The results obtained and the graph (Fig. 1) show that:

(1) The ratio is 1 : 1 in maize and 43 : 57 in nutmeg, though the numbers are perhaps too small to justify definite conclusions.

(2) In maize, emergence of adults ceased after 4 weeks, whereas in nutmeg it continued till the end of the eighth week.

(3) As seen in the graph, the maximum life cycle in nutmeg is nearly double that in maize, but the minimum life cycle (as mentioned, p. 570) varies less, being 29 days in maize and 38 in nutmeg.

(4) The number of emerging adults shows that the insect is better adapted to maize than to nutmeg.

(5) In the later periods of emergence, the number of the males becomes higher than that of the females whether the ultimate ratio is 1 : 1 or not.

(6) Among the emerging adults there are a very few abnormally small in size which usually emerge very late and are not confined to one sex.

(3) *Sexual maturity.*

Sexual maturity of the male and female is not reached at the same time, the male being always mature before the female. By sectioning or making a smear of the testis in the first 2 days after emergence, it is found that no mature spermatozoa are present; they appear only on the third day and, although mature, are few in number and grouped together. The testis in the first 3 days after emergence is small, rounded and without the seven or eight follicles which are present in fully mature males. The rest of the reproductive organs do not differ from those in the mature male.

The ovary of the female in the first 3 days after emergence is without the vitellarium or the portion of the ovarioles which contains the developing eggs (Fig. 3). The germarium extends from the filaments to the paired oviduct. The ovary in this immature period is very small but,

apart from its small size and the lack of the vitellarium, does not differ from the ovary of the mature female (Fig. 2). In the majority of cases, small oocytes or eggs are budded off from the germarium on the fourth day. The eggs are never mature before the sixth day after emergence. Thus, sexual maturity of the male is usually 3 days earlier than that of the female.

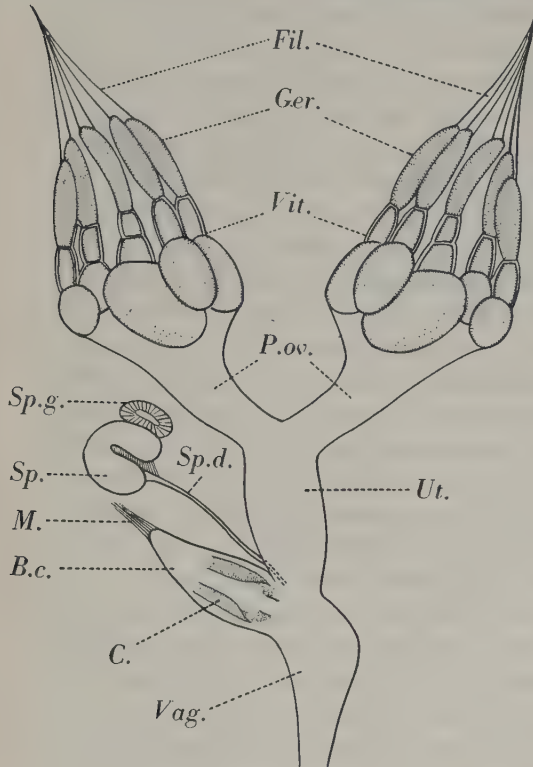


Fig. 2.

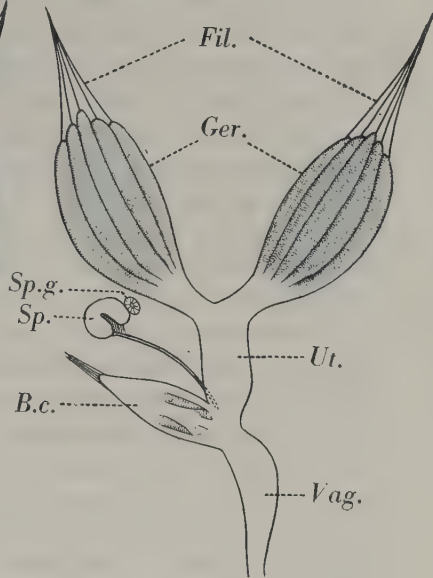


Fig. 3.

Figs. 2 and 3. Mature and immature reproductive organs of the female. $\times 25$ (approx.). B.c., bursa copulatrix; C., internal and lateral chitinisation in the bursa; Fil., filaments; Ger., germarium; M., muscles; P.ov., paired oviducts; Sp., spermatheca; Sp.d., spermathecal ducts; Sp.g., spermathecal gland; Ut., uterus; Vag., vagina; Vit., vitellarium.

Autuori, in his description of *Araecerus fasciculatus*, states that both sexes are sexually mature 2-3 days after emergence.

(4) Fertilisation and its effect on egg-laying.

With both sexes fertilisation usually takes place 6 days after emergence. Autuori records it "2-3 days after emergence when the adults are sexually mature", while Van der Goot gives 11-13 days after emergence

in the "Tephrosia beetle". The unfertilised female does not resist the male, especially if the sexes have been kept separate for a fortnight or more. Before fertilisation takes place, the male follows the female walking. The writer has not seen the small jumps which Autuori mentions. During fertilisation, the male is not in close contact with the female. The fore-legs are on either side of the posterior part of the abdomen of the female, but not touching it, and the pygidium of the female is opposite the metasternum of the male. The female does not change its normal position and thus does not raise the posterior part of its abdomen as Autuori maintains. The ejaculatory duct is protruded, distended into a glistening tube which becomes more or less colourless. Its anterior part enters into the female between the pygidium and the seventh sternite which may be seen widely separated. No part of the female genitalia is seen. A greater part of the male genitalia is external during fertilisation. Both the dorsal and the ventral lobes of the median plate are exerted and maintain their relative position. The ejaculatory duct is everted between them. The posterior part of the tegmen is exerted and serves as a further ventral support to the base of the ejaculatory duct.

Fertilisation (at laboratory temperature, about 18° C.) takes from 6.5 to 8 min., as determined from twenty observations. If, for some reason, the ejaculatory duct is withdrawn before the minimum period of fertilisation, it will be everted again instantaneously; the time taken before and after its temporary withdrawal does not exceed 8 min. Directly after fertilisation, the flagellum may be seen retracting into the ejaculatory duct while the latter is invaginating. The invagination does not, however, take place at once. The tube shortens till it is nearly as long as the pygidium and then protrudes out slightly. It is retracted and protruded five or six times before it is all invaginated. Its glistening appearance is only noticeable during fertilisation, as it looks opaque while it is retracted.

The female under normal conditions is always fertilised more than once by the male. In order to determine whether it was necessary for the female to be fertilised more than once in order to lay fertile eggs, twenty virgin females which had been kept separate from the males for a fortnight were allowed to be fertilised only once by males of about the same age. Each female was then placed separately in a tube with grains of maize. Fifteen out of twenty females laid fertilised eggs till they died. The remaining five females laid only unfertilised eggs in the frass at the bottom of the tube (see p. 566). The eggs laid by the fertilised females varied considerably in number, the minimum being forty-two, while the

maximum reached 125 eggs. The average number was seventy-nine eggs.

It appears therefore that in most cases one fertilisation is sufficient for normal egg-laying.

(5) *Effect of age on fertilisation.*

Fertilisation is not affected by the age of the male or the female and may take place at any time during the period of sexual maturity and activity. The activity of the adults is remarkable except in the last 3 or 4 days of their lives. In this period the female does not lay any eggs and the male does not attempt to fertilise the female.

To show this, a hundred pairs of newly emerged males and females were used. Ten males and ten females were kept separate for a week; another lot, of the same size, was kept separate for a fortnight, and so on for 10 weeks. In every case, on bringing them together in small tubes with maize grains in equilibrium with 90 per cent. R.H., fertilisation took place and this was followed by egg-laying. All the insects of each sex did not, however, survive in every group. The experiment was carried as far as the tenth week because this is about the average length of life in the unpaired females. The longevity of paired adults in 90 per cent. R.H. is much less than 10 weeks.

(6) *Effect of delayed fertilisation on the rate of egg-laying.*

Using the fertilised females from the previous experiment, it was found that delayed fertilisation increases the rate of egg-laying, but this increase is not necessarily proportional to the period after which fertilisation has taken place. The maximum rate for the females that were separated from the males for only 1 week was 5.3 eggs per day, while the average number of eggs was about 2 per day. When fertilisation was delayed a fortnight, the maximum rate of egg-laying rose to 6.3, the average being 2.9 per day. With those kept unfertilised for 3 weeks, the maximum rate was 6.7 and the average 3.4 per day.

Further delay in fertilisation did not seem to increase further the rate of egg-laying. In the last three batches the females lived a maximum of 16 days after fertilisation and in all of them numerous eggs were found in the ovary. In nearly all cases, females die before they deposit all the eggs in their ovaries. It is probable that egg-laying by females in which fertilisation is delayed is affected by the age of the female and by the number of unfertilised eggs laid before fertilisation takes place.

It was noticed in experiments on egg-laying in different humidities (which will be mentioned later) that oviposition was continuous. The

rate is usually high in the third, fourth and fifth weeks in 90 and 100 per cent. R.H., in the second and third weeks in 80 per cent. R.H. and in the second in 60 and 70 per cent. R.H.

The previous results are tabulated as follows:

Fertilisation delayed...	1 week	2 weeks	3 weeks	4 weeks	From 5 to 10 weeks
Average daily rate of egg-laying	2	2.9	3.4	2.7	The rate is irregular and falls after 4 weeks
Maximum daily rate	5.3	6.3	6.7	5.6	Females do not live long after fertilisation

(7) *The egg-laying process.*

(Observations at about 18° C.) Egg-laying usually begins within half an hour of fertilisation but may be delayed as long as 6 hours. It occurs in both daylight and darkness and it was found by examining the grains of maize which had been with fertilised females for different periods in darkness that eggs were not laid any quicker than in daylight. In a few cases the female bores its ovipositor into the grains but without laying. Eight minutes is the average time taken to lay a batch of eggs. The female begins by feeling the endosperm of the grains with its mandibles without any actual biting or feeding, this preliminary step taking not more than 30 sec. The insect then turns the posterior end of the abdomen to the endosperm. The coxites and style of the ovipositor are protruded and begin to dig into the endosperm in a vertical direction. The long axis of the beetle itself remains nearly vertical to the grain in spite of any movements of the latter. The boring of the ovipositor is a piercing rather than a screwing motion, the ovipositor being partially drawn back and pushed out again periodically, till it settles into the grain. This takes most of the time. Almost immediately oviposition is over, the ovipositor is finally withdrawn. In all cases, this is followed by evidence of excitement on the part of the female. It moves its abdomen right and left several times for about 20 sec. as if it were dancing and then leaves the grain and resumes its normal condition. The times mentioned above were observed in the laboratory on grains of maize which had been in equilibrium with 90 per cent. R.H.

(8) *Unfertilised eggs.*

Unpaired females lay unfertilised eggs and it is remarkable to find that these are laid without any exception in the frass instead of inside the food. Unfertilised eggs are very few in number and are laid at irregular intervals. The maximum number of unfertilised eggs laid by one female on maize in equilibrium with 90 per cent. R.H. was 21. The

observations were made on twenty-five unpaired females used directly after emergence from the pupae.

(9) *The incubation period.*

The incubation period, like the pupal period, does not vary much in different humidities. It is from 5 to 8 days at 27° C. Paired females were put on the food for 5 hours in different humidities ranging from 50 to 100 per cent. R.H. The eggs were taken out of the grains and assumed to be all laid in the third hour. In low humidities (up to 70 per cent. R.H.), very few eggs were found, but the observations made indicated that the maximum and minimum periods for incubation in different humidities differed only by a few hours. Van der Goot mentions that the egg incubation period of the "Tephrosia beetle" is 6-7 days, while Autuori, in an indirect way, indicates 6-9 days. Neither Van der Goot nor Autuori refer to the temperature or humidity.

The translucency or glistening of the egg is affected by the relative humidity of the atmosphere. In 50 per cent. R.H. it almost vanishes after 2 days; whereas in high humidities (from 80 per cent. R.H. upwards), it remains most of the time. The segregation of proteins is seen 2 days after the eggs are laid. In an unfertilised egg this is not noticed; it remains clear and homogeneous.

(10) *Egg-laying and humidity.*

Egg-laying is indirectly affected by the relative humidity of the atmosphere in which the paired females are placed. The three foods used, cacao beans, maize grains and nutmeg, absorb moisture from an atmosphere of a given humidity at different rates. Cacao beans have the most rapid absorptive powers, but finally absorb less moisture than maize. It is the water content of the food which has a direct effect on oviposition, and thus the relative humidity has an indirect effect. Females deposit their eggs on maize grains in equilibrium with 60 per cent. R.H. and to a much less extent in 50 per cent. R.H., but no eggs are deposited on cacao beans in equilibrium with 70 per cent. R.H. or less. Oviposition does not usually take place in cacao with water content less than about 7.5 per cent. Nutmeg lies about half-way between maize and cacao as far as oviposition is concerned. No eggs are laid on nutmeg which is in equilibrium with less than 60 per cent. R.H. In all cases egg-laying varies directly with the water content of the food concerned except when there is condensation or excess of water vapour in 100 per cent. R.H. If the 100 per cent. desiccator is not opened for a week or more, the adults of

both sexes become immobile either from the accumulation of CO_2 and water vapour or from both. As this occurs to a less extent in 90 per cent. R.H., it seems that CO_2 is not the chief cause. This condition increases the rate of mortality and if it continues all the adults die.

(11) *Method used for determining the number of eggs and viabilities at different humidities.*

For determining the maximum and average number of eggs laid under different conditions twenty-five newly emerged pairs of adults were used for each relative humidity at intervals of 10 per cent. between 50 and 100 per cent. R.H., except for 60 per cent. R.H., for which thirty pairs were used. Seven grains of maize were split longitudinally before they were used in order to make the search for eggs easy. Eggs were looked for weekly and, since the minimum incubation period is 5 days, some larvae were usually found with the eggs.

For determining the viability of eggs, several adults were placed on grains of maize in equilibrium with the respective humidities. The females were allowed to lay eggs for 3 days and then removed. The grains were examined for the larvae 9 days after all the eggs were laid, thus giving time for all the eggs to hatch. The maximum age of the eggs that do not hatch would be 12 days. It was found that the eggs after this time were still conspicuous in the endosperm of the grains.

An attempt was also made to discover the viability of exposed eggs in humidities from 60 per cent. R.H. upwards. This is not the natural condition for *Araecerus fasciculatus*, as not less than 98 per cent. of the

Table II.

Egg-laying and viability of eggs in different humidities on maize.

Relative humidity %	Per-centage water content of maize	Maximum number of eggs laid by 25 females in a week	Average number of eggs laid by 25 females in a week	Eggs inside the grains				Eggs exposed			
				Number of eggs used	Per-centage viability	Per-centage de-veloped but failed to hatch	Per-centage of eggs with no de-velop-ment	Number of eggs	Per-centage viability	Per-centage de-veloped but failed to hatch	Per-centage of eggs with no de-velop-ment
50	6.7	7	3	122	39.3	46.7	14	0	—	—	—
60	8.4	31	18	239	67	27	6	150	60.6	18	21.4
70	10.1	62	32	259	82.6	14.3	3.1	150	74	12	14
80	13.5	99	57	619	92.6	2.3	5.1	200	79	10	11
90	16.9	137	91	640	95	—	5	200	84	6	10
100	21.2	129	85	548	98	—	2	200	85	7	8

In order to get the large number of eggs required, the experiments on viability had to be repeated with comparatively small batches of eggs. In all these experiments the variation in the viability of eggs was comparatively small, especially for eggs inside the grains. It should be noted that the percentages of exposed eggs with and without embryonic development in 60 and 70 per cent. R.H. are approximate, as in a few cases it was very difficult to make a sharp distinction between the two.

total number of eggs are laid inside the grains. As already mentioned, however, unpaired females lay all their eggs without any exception in the frass. Some or all of the few eggs laid in the frass by paired females hatch.

The figures obtained are given in Table II.

(12) *General notes on egg-laying and viability of eggs in different humidities.*

At 60 per cent. R.H. there is hardly any feeding by adults placed with cacao; the mortality follows practically the same curve as if they were starved or without any food. Oviposition occurs in nutmeg in equilibrium with this humidity but it is extremely difficult to obtain any accurate idea about the maximum or average number of eggs laid. Out of the thirty paired females used to determine the number of eggs, two died in the second week and did not lay any eggs; and a marked variation in the capacity of the insects for egg-laying was observed. The largest number of eggs was laid in the second and third week, the maximum rate of egg-laying being 2.4 eggs per day. Only about 2 per cent. of the eggs were laid in the frass, and although the conditions at this humidity were not favourable for oviposition, yet the number of eggs laid in the frass was not more than at higher humidities.

There is a marked difference in oviposition and viability at 60 and at 50 per cent. R.H.; and since eggs laid in maize in equilibrium with 50 per cent. R.H. do not succeed in passing to the adult stage, it may be taken that 60 per cent. R.H. is the minimum for development at 27° C. when maize is used.

At 70 per cent. R.H. cacao is still unfavourable for oviposition, and this humidity could safely be advised as the limit in cacao stores, even in tropical and subtropical countries. As at 60 per cent. R.H., females vary much in their egg-laying capacity. At both humidities egg-laying ends more or less abruptly. This is noticeable when comparing the number of eggs laid in the last 2 weeks in the life of the female. 80 per cent. R.H. is favourable for oviposition in maize grains. It also appears to be favourable in nutmeg but to a less extent in cacao, as deduced from the number of adults emerging from the two latter when the number of paired females, their age and the period they are allowed to lay their eggs are the same and assuming that the mortality of the larvae and pupae is about equal in nutmeg and cacao.

Although this humidity is fairly high, there is 13.6 per cent. difference in the viability of eggs inside the grains and exposed. Maize and nutmeg may become slightly mouldy at this humidity but this does not seem to

affect oviposition and the development of the larvae as at 100 per cent. R.H.

90 per cent. R.H. is very favourable for rearing the beetle on any food. It is only when the food gets too mouldy that development is retarded. It is interesting that at this humidity none of the eggs deposited inside the food started their embryonic development and failed to hatch as at lower humidities.

100 per cent. R.H.¹ is very favourable provided there is no condensation of water vapour and the food does not get mouldy. If the desiccator is opened for a few seconds even every 3 days, the toxic effects on the insects associated particularly with distilled water are not found. When twenty-five paired females were placed in this humidity for egg-laying, all were found to be immobile at the end of the week, but nearly all regained their mobility when exposed for about 1 hour. In another experiment with twenty-five pairs, with a maximum age of 2 days, the desiccator was opened for about 20 sec. once every 3 days and it was noted that the insects were active all the time.

Forty eggs with a maximum age of 12 hours were placed at 100 per cent. R.H. in a desiccator which was only opened 8 days later. None of the eggs hatched, although they were allowed their maximum period. They all lost their translucency, became opaque and very soft when touched. Larvae and pupae are affected in the same way as the adults at this humidity.

The toxic effect does not necessarily appear within a week; it may be delayed. As in 90 per cent. R.H., no larvae failed to hatch and the viability of exposed eggs is about the same (at any rate when the desiccator is opened at intervals). As shown in Table II, oviposition and viability increase with increasing water content of the food.

(13) *The minimum length of the life cycle in different humidities and foods.*

In conducting these experiments, the three foods, maize, cacao and nutmeg, were used after they had reached equilibrium with different humidities ranging from 50 to 100 per cent. R.H. The paired adults used had a maximum age of 3 weeks and had all emerged in 80 and 90 per cent. R.H. They were allowed to lay eggs for 5 days, after which they were removed. In both maize and nutmeg every experiment was duplicated; in cacao the only duplicate was at 100 per cent. R.H. In cal-

¹ Obtained by the use of distilled water: usually 97-98 per cent. R.H.

culating the minimum length of the life cycle, all the eggs were considered to be laid on the third day.

The results may be tabulated as follows:

TABLE III.

The minimum length of the life cycle of Araecerus fasciculatus at 27° C. under different relative humidities and on different foods.

Food	60 % R.H. days	70 % R.H. days	80 % R.H. days	90% R.H. days	100 % R.H. days
Maize	57	51	43	35	29
Nutmeg	69	59	51	43	38
Cacao	—	—	66	45	37

The figures are also plotted in the graph (Fig. 4) and, by reference to the percentage moisture content of the foods at 27° C. (Table I), they indicate that the length of the life cycle varies inversely with the water content of the food. As the egg period (5–8 days) and the pupal period (6–7 days) do not vary greatly in different humidities, it is evidently the larval stages that are affected by the different degrees of humidity.

It has been already mentioned under "sex ratio" that in maize and nutmeg (100 per cent. R.H. and 27° C.) emergence lasts 4 and 8 weeks respectively. Consequently, the range of the life cycle in maize is from 29 to 57 days; while in nutmeg it is 38–94 days. Taking 12 days as the approximate period for the egg and the pupa, the range of the larval period is found to be 17–45 days in maize and 26–82 days in nutmeg. Under exposed (or partially exposed) conditions the range of the larval period in maize under the same temperature and humidity (27° C. and 100 per cent. R.H.) is 21–72 days.

In nutmeg which contains less water than maize (according to figures received from the Imperial Institute), the life-cycle periods run parallel to those obtained for maize (see Fig. 4).

70 and 80 per cent. R.H. in maize correspond to 80 and 90 per cent. R.H. in nutmeg as far as the minimum length of the life cycle is concerned. The same may be said about 60 per cent. R.H. in maize and 70 per cent. R.H. in nutmeg, as there is only 2 days' difference. At 100 per cent. R.H. the difference between the two foods is 9 days, whereas it is only 1 day between nutmeg and cacao.

There is a marked difference between 80 and 90 per cent. R.H. in the cacao curve which is not parallel to either the maize or the nutmeg curve.

Although at 80 per cent. R.H. in cacao the life cycle is totally different from that in nutmeg, yet in 90 and 100 per cent. R.H. the periods are similar.

It is evident that the length of the life cycle is not the same in different foods containing the same percentage of water.

For example, maize in equilibrium with 80 per cent. R.H. contains about 13.5 per cent. water and the minimum period is 43 days. About the same amount of water is found in cacao in equilibrium with 100 per cent. R.H. and the maximum period is only 37 days. It appears, therefore, that the water content of the food is not the only factor that affects the

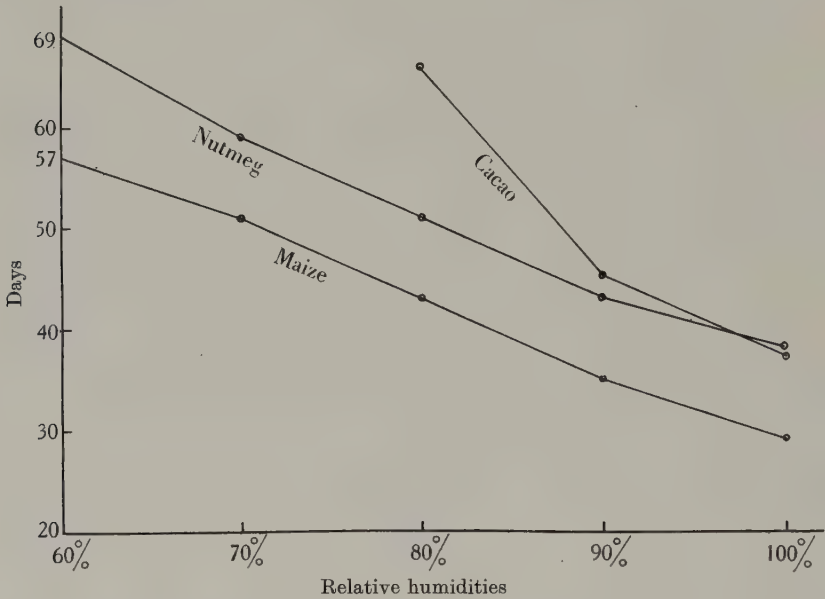


Fig. 4. Curve for minimum lengths of the life cycles in different humidities at 27°C. using maize, cacao and nutmeg.

length of the life cycle, as it seems that the chemical composition of the food is also concerned.

The pupal period here described as lasting from 6 to 7 days was stated by Lucas⁽¹⁵⁾ to be "12-15 days in the third stage". Reh⁽²¹⁾ probably copied Lucas by stating that "the pupal stage has been said to take about two weeks". Van der Goot⁽²⁸⁾ in his short description of the life history of the "Tephrosia beetle" refers to the pupal period as lasting 6-7 days. Autuori⁽¹⁾ mentions 6-9 days in the Brazilian coffee beetle. These authors do not refer to temperature or humidity.

(14) *Effect of low humidity on the pupa.*

In contrast to the larva, the pupal period does not differ in different humidities. It is the only stage in the life history of the insect which resists lower humidities. Of twenty newly formed pupae kept at 50 per cent. R.H., sixteen emerged, nine females and seven males.

(15) *The longevity of adults in different humidities.*

For the determination of the longevity of adults, 100 newly emerged beetles with a maximum age of 3 days were used for all humidities. One male and one female were placed together in a small tube ($2 \times \frac{2}{3}$ in.). The maximum longevity was determined only in maize. Besides the fifty males and fifty females used for every humidity, the twenty-five pairs used for determining the number of eggs were considered also. In cacao, the intention was only to determine how many adults died in 4 weeks and thus to discover which relative humidity was most favourable. The same number of adults and the same size of tube as for maize were used.

For nutmeg, the same size of tube could not be used owing to the shape of the nutmeg. Fifty males and fifty females were placed in a big jar (the size of an ordinary jam pot) and the mortality determined every week for the first month.

The results are given in Tables IV and V.

TABLE IV.
Longevity of adults in maize.

R.H. %	Maximum age in days		Average age in days		Remarks
	Males	Females	Males	Females	
50	28	27	12	16	40 unpaired adults were used in addition, with nearly similar results
60	40	35	28	23	50 % males and 80 % females died after 4 weeks
70	56	48	37	31	36 % males and 62 % females died after 4 weeks
80	71	62	50	47	24 % males and 28 % females died after 4 weeks
90	134	86	69	58	8 % males and 16 % females died after 4 weeks
100	Mouldy		Mouldy		

The length of life in nutmeg is less than in either maize or cacao; but as the method adopted in nutmeg was quite different from the other two, a close comparison cannot be made. A mouldy culture of nutmeg in 100 per cent. R.H. leads to quicker mortality than mouldy maize or cacao.

TABLE V.

Longevity of adults in cacao.

R.H. %	Remarks on longevity				
50	Starve, all dead after 14 days except 3 that survived till end of 3rd week				
60	Starve, only 2 survived till end of 3rd week				
70	58 % starve (die after 14 days); 90 % die after 3 weeks. Maximum age 29 days (male)				
80	26 % females and 18 % males die after 4 weeks				
90	12 %	"	8 %	"	"
100	14 %	"	12 %	"	"

(16) *Starvation of adults in 60, 80 and 100 per cent. R.H.*

50 males and 50 females about 3 weeks old were removed from maize at 90 per cent. R.H. and used for each of these humidities. Two males and two females were placed together in one small tube ($2 \times \frac{2}{3}$ in.). It was intended to determine whether mortality would increase in lower humidities when the food factor was eliminated.

The results may be tabulated as follows:

TABLE VI.

R.H. %	No. dead after 7 days		Total	No. dead after 9 days		Total
	Male	Female		Male	Female	
60	14	12	26	40	32	72
80	14	8	22	38	32	70
100	14	10	24	38	26	64

The following conclusions may be drawn from Table VI:

(1) The insects during the period of starvation do not respond to the differences in humidities in the first week.

(2) Mortality is usually more rapid in the males than it is in the females.

(3) The maximum starvation period was 14 days in both 60 and 100 per cent. R.H.

Since there is only a small difference between the mortality in 60 and 100 per cent. R.H., it appears that the atmospheric humidity is not itself important so far as the longevity of the insects is concerned, but acts through its effect on the food. The insects become very sluggish and inactive 3 days before they die.

SUMMARY.

1. The time necessary for maize and cacao (the two important foods of *Araecerus fasciculatus*) to come in equilibrium with atmospheric humidities between 50 and 100 per cent. R.H. have been determined. The same determination has been made more roughly for nutmeg.

2. Under ideal conditions (maize at high humidities) the sex ratio of *A. fasciculatus* is about 1 : 1. On nutmeg (which appears to be a less suitable food) more females than males are produced.

3. At 27° C., the male is mature 3 days and the female 6 days, after emergence.

4. Fertilisation takes place at 6 days after emergence, and lasts 6.5–8 min., at 27° C. Females are normally fertilised more than once, but once is enough to render all the eggs fertile.

5. Females in which fertilisation is delayed lay more rapidly when once they are fertilised. The effect gradually increases for delays of 1, 2 and 3 weeks and after that ceases.

6. Oviposition in maize is described. It takes on the average 8 min. (at 18° C.) whether in light or darkness. Unfertilised eggs are laid loose and are not inserted into the food.

7. The incubation period is 5–8 days at 27° C. at all humidities between 50 and 100 per cent. R.H.

8. The maximum number of eggs was laid by groups of 25 females on maize (at 27° C.) at R.H. 90 and 100 per cent. At lower humidities the number laid was less and the viability smaller. 60 per cent. R.H. is about the limit at which the life cycle can be carried on with maize and nutmeg, 80 per cent. R.H. for cacao.

9. For maize and nutmeg, the minimum length of the total life cycle varies inversely with the relative humidity of the atmosphere, the period always being about 10 days less on the former. For cacao there is a similar relation, but the curve is not parallel to those for maize and nutmeg. On maize the minimum life cycle at 27° C. varies from 57 days at 60 per cent. R.H. to 29 days at 100 per cent. R.H., the variation occurring only in the period spent as a larva.

10. The pupa is the only stage which can survive humidities lower than 60 per cent. R.H.

11. On maize, the adults live 27–28 days at 50 per cent. R.H. and 86–134 days at 90 per cent. R.H. (at 100 per cent. the food becomes mouldy). On cacao, few live more than 20 days at humidities less than 80 per cent. R.H. The reduction in length of life is principally due to failure to feed at lower humidities, for when starved, the beetles live about the same time at all humidities.

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THE PROBLEM OF THE EVALUATION OF ROTENONE-CONTAINING PLANTS

I. *DERRIS ELLIPTICA* AND *DERRIS MALACCENSIS*

BY F. TATTERSFIELD, D.Sc., F.I.C.

AND

J. T. MARTIN, B.Sc., PH.D., A.I.C.

(*Department of Insecticides and Fungicides, Rothamsted
Experimental Station, Harpenden, Herts.*)

(With 9 Text-figures.)

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INTRODUCTION.

THE increase in the production and use of *Derris* root and other rotenone-containing plants as insecticides, have given rise to a demand for methods for their chemical evaluation. Several have been proposed for this purpose, but without meeting with general acceptance. This is due to the fact that more than one chemical compound are responsible for the insecticidal potency of these plants, while the amounts of the active principles may vary within wide limits. Of these active principles,

rotenone is readily obtained from most samples and is known to be highly toxic to insects and fish. Of the other crystalline derivatives isolated, deguelin, tephrosin and toxicarol are also toxic to insects, but as the potencies of these compounds are greatly inferior to that of rotenone, it has been suggested that their presence might be ignored as contributory factors to toxicity, and that the amount of rotenone present might well be a measure of activity. It has been known for many years, however, that the resins after the isolation of rotenone were still highly effective insecticides, and evidence suggests that deguelin and toxicarol are present in the root in some other form. Clark⁽³⁾ when describing its isolation stated this view with respect to deguelin. Evidence has recently been produced by Takei and his co-workers^(8, 9) that tephrosin is produced from deguelin in the process of isolation. The relative activities, therefore, of these isolated crystalline derivatives may have little bearing upon the toxicities of their precursors in the root.

The determinations of the ether extract and its methoxyl content have also been proposed as a possible means of evaluation. In addition, the recent work of Takei⁽⁹⁾ on the separation of the dehydro compounds suggests that the determination of these compounds may be important, and it would seem that a critical discussion of the possibility of evaluating *Derris* and other rotenone-containing plants by chemical means would be timely. No chemical method, however, can be regarded as valid unless the results given by it can be correlated with insecticidal activities obtained by biological tests. It must also be applicable to all kinds of rotenone-bearing plants in use.

The evaluation of an insecticide is primarily a biological problem, and for this purpose data which connect mortality or paralysis with the dosage of, or the time of exposure to the poison are frequently used. Biological tests demand a high measure of technical skill, and an abundant supply of suitable insects, free from parasites and disease, are required if reliable quantitative data are to be obtained. Suitable biological material may only be available at certain limited periods of the year, and in addition to the normal differences in resistance shown between individual insects, the mean resistance of the insect population may change with time or environmental conditions. As a consequence, comparative tests should be carried out at intervals of time not too widely separated and preferably on the same day. Changes in the powers of resistance of the test insects might be detected by the use of a standard preparation of an insecticide, but there is a risk that this might gradually change in potency and become unreliable. Biological tests are difficult to express on a

quantitative basis, and their results require a statistical analysis if valid comparisons are to be made. Chemical and physical tests, on the other hand, are not subject to many of these difficulties. If the active principle can be determined with a degree of accuracy commensurate with that of the biological trials, in general, such a determination will give a basis for standardisation. In certain cases, however, a determination of this kind cannot readily be made, or there may be present several compounds of different degrees of activity. The problem is then more difficult, and may necessitate the determination of some factor or factors, the validity of which will depend upon the degree of correlation shown with the biological potencies of a number of samples of the insecticide.

We propose in this paper to consider, in a preliminary way, some of the methods which have been tentatively suggested and to compare the results, obtained with samples of *Derris* of known origin, with the insecticidal performance of the samples. In these trials *Aphis rumicis* has been used throughout as a test subject. We feel that the use of only one species of insect is open to criticism, but the data obtained have enabled us to rule out certain chemical determinations as inadequate as a means of evaluation. We have made no attempt to criticise the methods of analysis, or to elaborate new ones.

In our view there are two criteria of the validity of a chemical method for evaluating an insecticide.

(1) The chemical determinations should be closely correlated with the insecticidal activities of the samples. Theoretically, at the same concentration expressed in terms of such a chemical determination, two or more samples should show the same toxic effect. In actual practice, greater reliance can be placed upon the comparisons, if tests are made at a number of concentrations of each sample, and the characteristic curves, relating toxic effect to concentration, determined. It is then possible to ascertain, by suitable statistical transformations and analyses⁽¹⁾, how closely the curves approximate to each other in coincidence. The nearer they are to coincidence the more dependable the factor estimated will be as a basis for evaluation. If stocks of insects could be raised, the variation in the resistance of which was approximately constant about a certain mean, it would be possible to construct a standard curve, the validity of which would increase with each experiment carried out. Comparisons could then be made with this standard. So far, however, we have not been able to construct such a curve, and for purposes of comparison we have relied upon tests carried out on two samples on the same day.

(2) In the case of an insecticide such as *Derris*, which may lose its activity, a completely valid method of analysis should be able to trace out the loss with time. The characteristic dosage-mortality curve for a degenerated sample, when concentrations are expressed in terms of the value determined, should approximate in slope and position to that of the undeteriorated sample.

Georgi and Teik⁽⁵⁾ have discussed the difficulties which may arise in the sampling and grinding of *Derris* roots. The points raised by them can only be emphasised by us. Owing to the heterogeneous nature of the baled material, it is desirable that an agreed and uniform sampling technique should be adopted. Georgi and Teik (*loc. cit.*) have pointed out the importance of the recovery of the finer material during grinding, the loss of which may lead to erroneous results. It has been our experience, that unless a ground sample is practically impalpable, there is a tendency towards segregation into rich and poor fractions. Thus a sample should be very thoroughly mixed before any portion of it is abstracted for analysis.

EXPERIMENTAL.

Through the kindness of the Director of Agriculture, Straits Settlements and Federated Malay States, seven samples of roots of certain species of *Derris* were sent to us in hermetically sealed tins. The roots were received cut up into small pieces but not ground. Duplicate samples were retained in Malaya for analysis. Owing probably to differences in the sampling and grinding techniques, the analyses first carried out in Malaya and at Rothamsted gave discordant results. As a consequence, portions of the material ground in England to a relatively fine powder were returned to Malaya, and analysed there for moisture content, crude and recrystallised rotenone and ether extract. It was then found that the results of analysis carried out at the two centres showed fairly good agreement.

Determination of moisture content, ether extract and rotenone.

The methods employed differed somewhat in the two countries. In Malaya, moisture was determined by the xylene method and the ether extract by extracting 5 gm. with ether, the extract being dried to a constant weight in a steam oven. The crude rotenone was estimated by extracting 50 gm. in the cases of samples 1-6, and 25 gm. in the case of No. 7, with carbon tetrachloride, followed by the separation of the carbon tetrachloride compound. The recrystallised rotenone was then deter-

TABLE I.
*Results of analysis of samples of Derris for ether extract
 and methoxyl content.*

No.	Sample	Moisture	Weight of root extracted, and oven used for drying ether extract	Ether extract	Methoxyl content	Moisture-free basis	
						Ether extract	Methoxyl content
1	<i>D. elliptica</i> , tuba puteh, W. 146, 22 months	6.89	5 gm., electric	10.70		11.52	
		—	†2.5 gm., electric	10.75			
		9.10	5 gm., steam	10.14	1.43	10.89	1.54
2	<i>D. elliptica</i> , tuba puteh, W. 148, 28 months	7.00	5 gm., electric	7.12		7.69	
		—	†2.5 gm., electric	7.18			
		8.75	5 gm., steam	6.77	0.85	7.28	0.91
3	<i>D. elliptica</i> , Sarawak creeping, W. 149, 22 months	6.25	5 gm., electric	7.62		8.35	
		—	†2.5 gm., electric	18.16		19.34	
		7.90	5 gm., steam	18.10	2.58	18.31	2.75
4	<i>D. malaccensis</i> , Sarawak erect, W. 147, 22 months	5.86	5 gm., electric	17.17		20.52	
		—	†2.5 gm., electric	16.26		17.27	
		8.40	5 gm., steam	15.45	2.16	16.41	2.29
5	<i>D. malaccensis</i> , Sarawak erect, W. 151, 28 months	6.31	5 gm., electric	16.84		18.38	
		—	†2.5 gm., electric	19.24		20.54	
		8.00	5 gm., steam	18.63	2.64	19.88	2.82
6	<i>D. polyantha</i> , W. 150, 48 months	6.46	5 gm., electric	20.12		21.87	
		—	†2.5 gm., electric	12.27		13.14	
		8.45	5 gm., steam	12.31	1.65	12.31	1.76
7	Tuba root* from Paya Lebar, Singapore, W. 153	5.62	5 gm., electric	12.64		13.81	
		—	†2.5 gm., electric	25.08		26.58	
		8.00	5 gm., steam	25.09	3.50	25.47	3.71

* Samples from Paya Lebar usually have same botanical characteristics as *D. elliptica*, tuba puteh. Sample No. 7 is probably *D. elliptica*.

† Extract made with sodium-dried ether.

The figures in italics were determined in Malaya, the remainder at Rothamsted (see p. 581).

The figures for methoxyl contents are the means of several closely agreeing determinations.

mined by the method outlined by Georgi and Teik (5). All samples prior to extraction were mixed with B.D.H. sand, washed free from acid.

Our procedure was as follows: Moisture was determined by heating in an electric oven kept at 100° C. until constant weight was reached. The ether extract was determined by extracting 5 gm., mixed with acid-washed sand, in a Soxhlet apparatus, the extracted matter being dried to constant weight in an electric oven kept at 100° C. Crude rotenone was determined by extracting 50 gm. with ether, the solvent being taken off in carbon dioxide and finally in partial vacuum. The residue was dissolved in carbon tetrachloride,¹ and after concentrating to 25 c.c. the cooled solution was seeded and placed in an ice-chest

¹ With some samples it was necessary at this stage to filter the test solutions through cotton-wool, in order to separate small quantities of insoluble matter.

TABLE II.

Rotenone content of seven samples of Derris root.

No.	Sample	Solvent used in extracting	Rotenone* (crude) %	Rotenone† (purified) %	Moisture-free basis	
					Rotenone (crude) % (mean)	Rotenone (purified) % (mean)
1	<i>D. elliptica</i> , tuba puteh, W. 146, 22 months	Ether	{3.46 3.26}	{2.92 2.68}	3.61	3.02
		Trichlorethylene (C and B)	3.22	2.85	—	—
		<i>Carbon tetrachloride</i>	3.62	3.11	3.98	3.42
2	<i>D. elliptica</i> , tuba puteh, W. 148, 28 months	Ether	{1.62 1.67}	{1.37 1.45}	1.77	1.52
		Trichlorethylene (C and B)	1.68	1.50	—	—
		<i>Carbon tetrachloride</i>	1.63	1.35	1.79	1.48
3	<i>D. elliptica</i> , Sarawak creeping, W. 149, 22 months	Ether	{5.07 5.09}	{3.95 3.72}	5.42	4.09
		Trichlorethylene (C and B)	4.73	4.32	—	—
		<i>Carbon tetrachloride</i>	5.25	4.00	5.70	4.34
4	<i>D. malaccensis</i> , Sarawak erect, W. 147, 22 months	Ether	{2.64 2.76}	{1.96 2.03}	2.87	2.12
		Trichlorethylene (C and B)	2.43	1.95	—	—
		<i>Carbon tetrachloride</i>	3.18	1.99	3.47	2.17
5	<i>D. malaccensis</i> , Sarawak erect, W. 151, 28 months	Ether	{2.52 2.56}	{1.79 1.87}	2.71	1.96
		Trichlorethylene (C and B)	2.60	2.0	—	—
		<i>Carbon tetrachloride</i>	2.51	1.72	2.73	1.87
6	<i>D. polyantha</i> , W. 150, 48 months	Ether	{4.61 4.61}	{3.69 3.76}	4.93	3.98
		Trichlorethylene (C and B)	4.40	4.10	—	—
		<i>Carbon tetrachloride</i>	4.70	4.03	5.13	4.40
7	Tuba root from Paya Lebar, Singapore, W. 153	Ether	{9.53 9.43}	{8.58 8.43}	10.05	9.02
		Ether (sodium dried)	{9.59 9.49}	{8.09 8.20}	10.11	8.63
		Carbon tetrachloride	8.43	7.36	—	—
		Chloroform	{9.59 9.20}	{8.36 8.28}	10.45	8.82
		Trichlorethylene	8.56	7.87	—	—
		<i>Carbon tetrachloride</i>	9.21	7.82	10.11	8.50

* Crude rotenone was in all cases determined as the carbon tetrachloride derivative.

† Purified rotenone was determined in the Cahn and Boam method (C and B) by triturating with rotenone-saturated alcohol, and in all other cases by Georgi's method.

The figures in italics were determined in Malaya, the others at Rothamsted. The differences in the methods are described on p. 581 *et seq.*

The figures in brackets are duplicates carried out at Rothamsted.

overnight. The precipitated rotenone-carbon tetrachloride complex was separated, washed with the smallest possible quantity of the ice-cold carbon tetrachloride, allowed to stand overnight and weighed. The crude product was dissolved in a measured quantity of hot alcohol (usually 50 c.c.), cooled in an ice-chest, and then kept at 20° C. for some hours. The crystals were filtered off at the pump in a weighed Gooch crucible, washed with a little ice-cold alcohol, and finally dried in a vacuum desiccator over calcium chloride and weighed as rotenone (the product containing only a trace of chlorine). The percentage of purified rotenone

was calculated, an allowance (0.2 gm. rotenone per 100 c.c.) being made for the rotenone retained by the alcohol.

The species and ages of the samples dealt with, together with the analytical data, are given in Tables I and II.

Examination of results.

A consideration of Tables I and II shows that the analyses of each sample carried out under exactly similar conditions give results in good agreement (*e.g.* the figures bracketed together), but that there is some departure from agreement when independent investigators have used methods not precisely of the same kind. Thus, taking *seriatim* the items estimated:

(1) *Moisture content.* The results obtained in Malaya, using the xylene method, cannot be correlated with those obtained in England using an electric oven, owing to the ease with which finely ground *Derris* root in Malaya absorbs moisture from the atmosphere, resulting in a higher moisture content. While, therefore, it has not been possible to confirm the contention of Georgi and Teik⁽⁵⁾ that the xylene method gives higher results than the electric oven method, the importance of agreeing upon a standard method for moisture determination must not be overlooked.

(2) *Ether extract.* The methods of determination were almost identical as far as the extraction itself was concerned, but differed in that an electric oven was used for drying the extract at Rothamsted and a steam oven in Malaya. In every case the Malayan results are slightly higher than the mean Rothamsted results, the ratios on a moisture-free basis being: No. 1, 1 : 1.06; No. 2, 1 : 1.085; No. 3, 1 : 1.06; No. 4, 1 : 1.065; No. 5, 1 : 1.065; No. 6, 1 : 1.05; No. 7, 1 : 1.06; with a mean ratio of 1 : 1.065. The only sample to show a discrepancy is No. 2. The weight of ground root extracted was in each case 5 gm. At a later date portions of 2.5 gm. were extracted by us with sodium-dried ether, and the extracts weighed after drying to constant weight in an electric oven. The percentage weight of extract was in each case markedly lower, the figures being as follows on a moisture-free basis: No. 1, 10.9; No. 2, 7.3; No. 3, 18.3; No. 4, 16.4; No. 5, 19.9; No. 6, 12.3; No. 7, 25.5. The ratios of the mean values obtained by us when 5 gm. were extracted with ordinary ether (*not* dried over sodium) to those obtained using 2.5 gm. and sodium-dried ether were: No. 1, 1.06; No. 2, 1.05; No. 3, 1.05; No. 4, 1.06; No. 5, 1.03; No. 6, 1.07; No. 7, 1.08; with a mean ratio of 1.06. The later extracts were carried out for the purpose of determining the methoxyl content, shortly before the biological trials. Owing to the

comparative constancy of the ratios, the deductions drawn as to the relative toxicities are not invalidated. It is obvious, however, that slight changes in the technique used may make considerable differences in the determination of the matter extracted by ether.

(3) *Rotenone*. Although different solvents were used in the extractions, the rotenone determinations are on the whole in fairly good agreement, but there is no assurance that all the rotenone present was obtained in crystalline form. The determination of rotenone will probably always be a matter of importance in assessing the value of *Derris* root, owing to its known high toxicity to insects. The samples analysed indicate that the ratio of the amount of ether extract to rotenone content is higher in *D. malaccensis* than in *D. elliptica*, but that both of these values may vary within wide limits in each species.

In addition to the above, the methoxyl content and the yield of the mixture of the dehydro compounds were determined. These values and the data used for purposes of comparison with the insecticidal results are given in Table III.

TABLE III.

Analytical data used for determining the concentrations used in insecticide trials. All figures are expressed as percentages of ground root, and not on a dry-matter basis.

No.	Sample	Rotenone (crude)	Rotenone (recrys- tallised) (Georgi)	Ether* extract	Methoxyl	Dehydro mixture Takei separation from 5 gm. ether extract	Dehydro (new separ- ation) + recovery from filtrate	Recrys- tallised rotenone + dehydro mixture in residual resin	Rotenone† + deguelin (Gross and Smith)
1	<i>D. elliptica</i> , tuba puteh, W. 146, 22 months	3.36	2.80	10.1	1.43	6.12	6.41	6.61	5.0-7.5
2	<i>D. elliptica</i> , tuba puteh, W. 148, 28 months	1.65	1.41	6.8	0.85	3.46	3.53	3.38	3.75
3	<i>D. elliptica</i> , Sarawak creeping, W. 149, 22 months	5.08	3.83	17.2	2.58	10.18	10.75	11.23	10-15
4	<i>D. malaccensis</i> , Sarawak erect, W. 147, 22 months	2.70	2.00	15.4	2.16	5.33	6.88	5.82	5-7.5
5	<i>D. malaccensis</i> , Sarawak erect, W. 151, 28 months	2.54	1.83	18.6	2.64	6.85	7.90	7.14	10.0
6	<i>D. polyantha</i> , W. 150, 48 months	4.61	3.73	11.5	1.65	6.75	6.92	7.06	7.5-10.0
7	Tuba root from Paya Lebar, Singapore, W. 153	9.48	8.51	24.0	3.50	14.70	15.41	15.22	15-20

* 2.5 gm. of root extracted with sodium-dried ether.

† Incorporated for purpose of comparison.

Determination of methoxyl content.

This value was determined on the extract from 2.5 gm. obtained by the use of sodium-dried ether. After evaporation of the ether in an electric oven kept at 100° C., the methoxyl content was determined by the method of Clark (4), which we found both accurate and expeditious. The values obtained appeared to be closely correlated with the weights of the ether extract. The ratios of methoxyl content to ether extract were: No. 1, 0.142; No. 2, 0.125; No. 3, 0.150; No. 4, 0.140; No. 5, 0.142; No. 6, 0.143; No. 7, 0.146. The only sample to differ materially from the average of 0.141 is No. 2.

Determination of the dehydro derivatives of the active principles.

Takei (8,9) has shown that, by passing oxygen through alkaline alcoholic solutions of rotenone or deguelin, these compounds are converted to hydroxy derivatives, the rotenolones and deguelinols, and that by subsequent dehydration with alcoholic sulphuric acid, the corresponding dehydro derivatives are formed. The reaction is claimed to be practically quantitative. By hydrogenation in alkaline solution and appropriate treatment, the rotenone derivative can be separated from the deguelin derivative, the former giving rise to iso-dihydro-dehydro-rotenone which is soluble in alcoholic alkali, whereas the dehydro-deguelin remains unchanged.

The method for the determination of the dehydro derivatives as described by Takei (*loc. cit.*) is as follows: 5 gm. of the resin are dissolved in 150 c.c. of alcohol, and 3 gm. of 5 per cent. caustic soda are added. Oxygen is passed through the solution for half an hour at the rate of 150 c.c. per min. (air can be used, but the period should be extended for 1.5–2 hours). The reaction product is then acidified with 15 gm. of 50 per cent. alcoholic sulphuric acid, and 130 c.c. of alcohol are distilled off on the water-bath during a period of about half an hour. The residue is refluxed for a further hour. When cool, the mixture is poured into 500 c.c. of water and shaken in a separating funnel with 200 c.c. of ether. The dehydro derivatives separate as fine crystals in the ether layer, the resinous and tarry matter passing into solution. The whole is filtered through a weighed Gooch crucible, the crystalline mass washed with 10 c.c. of methyl alcohol, and subsequently weighed after drying at 100° C. The melting-point of the mixed crystals was found to lie between 200 and 215° C. A further small quantity of the dehydro compounds can be recovered from the filtrate.

Since the yield of dehydro derivatives is claimed to be a measure of the rotenone plus deguelin present in a sample, we considered it advisable to ascertain the correlation between the yield of these compounds and toxicity. A preliminary study was therefore made with the seven samples used in our biological tests. Certain modifications were made in Takei's method and three modes of separation employed.

(1) We found that in some of the samples much less rotenone could be crystallised out from ether (as recommended) compared with the amount separated by the Jones method (7) from carbon tetrachloride. 5 gm. of the ether extract, without a preliminary separation of rotenone, was therefore put through the Takei process as described and the yield of crystals weighed. They were rather dirty yellow in colour and usually had a melting-point of just above 200° C. The yield was expressed as a percentage of the root.

(2) It was found that recrystallised rotenone, when put through the Takei process, gave a yield of the order of 80 per cent. of dehydro-rotenone. It was clear that there was a possibility of loss in the solvents used for the final separation, from which, indeed, in the case of rotenone, the balance of 20 per cent. could be recovered. We therefore attempted the separation in the following manner. After the dehydration process with alcoholic sulphuric acid, the residue in the flask was cooled for some hours in the ice-chest, the crystals filtered through a Gooch crucible and washed successively with a little ice-cold ether, followed by 100 c.c. of distilled water, and finally with a few c.c. of ice-cold methyl alcohol. The crystals were dried at 100° C. and weighed. The filtrate was then separated by adding 400 c.c. of distilled water and 200 c.c. of ether. In some instances a further yield of crystals was obtained, which, after washing with a little ice-cold methyl alcohol, was weighed, the weight being added to that of the main crop. The results are shown in Table IV.

TABLE IV.

Weight of dehydro derivatives showing recovery from filtrate.

Sample	Dehydro derivatives main crop %	Recovery from filtrate %	Total yield %
No. 1, W. 146	6.33	0.08	6.41
No. 2, W. 148	3.53	Black resinous	3.53
No. 3, W. 149	10.75	"	10.75
No. 4, W. 147	6.51	0.37	6.88
No. 5, W. 151	7.72	0.18	7.90
No. 6, W. 150	6.92	Black resinous	6.92
No. 7, W. 153	15.16	0.25	15.41

(3) In the third modification the rotenone was determined by extraction with ether, separation as the carbon tetrachloride complex,

followed by recrystallisation from alcohol. The alcoholic mother liquor, containing a little rotenone and some resinous matter, was then used for dissolving the resin, which had previously been freed from carbon tetrachloride by distillation under reduced pressure followed by drying *in vacuo*. The yield of dehydro compounds from the alcoholic resin solution was then determined by the Takei process. In each case the carbon tetrachloride-free resin was weighed and the amounts of alkali and alcoholic sulphuric acid to be used calculated from this weight.

The yellow crystals showed a melting-point for the various samples in the region of 200° C. For the purposes of comparison with the biological trials the yield of rotenone was added to that of the dehydro mixture separated from the resin.

Gross and Smith's method (6).

Two methods of extraction were employed with the samples, of which the contents of rotenone and dehydro mixture were approximately known. The weights of *Derris* root extracted were so chosen that when their dry extracts were made up to 50 c.c. with acetone and diluted ten times, solutions containing approximately 1–2 mg. of rotenone plus deguelin per c.c. were obtained. In the first procedure used, the *Derris* root was extracted with acetone in a Soxhlet, the volume being adjusted to 50 c.c. An aliquot was diluted to one-tenth this concentration with acetone and 2 c.c. used for the test. In the second method extraction was with ether, the solvent being taken off and the residue dissolved in 50 c.c. of acetone, the subsequent procedure being as in (1).

It was found with these samples that the second method of procedure gave a better match than did the first. The results obtained were of the same order of magnitude as those given by the determination of the dehydro derivatives. They are given in Table III. The final analytical data used for determining the concentrations used in the biological trials are also given in Table III (p. 585).

INSECTICIDE TESTS.

The insecticide tests were carried out in the following way. A known weight of the powdered root was mixed with washed sand and extracted with ether in a Soxhlet apparatus, the ether being evaporated in a current of carbon dioxide and subsequently in a vacuum desiccator. The resin was dissolved in 5 c.c. of absolute alcohol and made up to 100 c.c. with 0.5 per cent. solution of saponin in water. Dilutions, the alcohol contents of which were adjusted to 5 per cent. by volume, were made from this

stock solution and used for the spraying trials, which were carried out in the apparatus described by one of us (11). Adult apterous females of *Aphis rumicis* were used for the tests. Control sprayings were carried out with 0.5 per cent. saponin solution containing 5 c.c. of alcohol per 100 c.c. Fifty insects, comprising five replicates of ten insects, were used for each concentration tested. After spraying, the insects without further handling were placed in tubes with a small amount of bean foliage. They were examined after 19–20 hours, a second examination being carried out 24 hours later. The results for the second examination were taken, and appear in the Tables V, VI, VII (pp. 593, 596, 600).

Method of expressing results.

It was necessary for our purpose, and for subsequent statistical treatment, that the dilutions used should cover a fairly wide range, giving at one end a high percentage of moribund and dead insects, and at the other an effect of the same order as the control. This limited the number of samples which could be tested on any one day. We therefore undertook on each occasion a comparison of two samples only. It is known that *Derris* is a relatively slow-acting poison to *Aphis rumicis*, and certain critical concentrations, although causing the eventual death of the insect, may not completely inhibit reproduction. Usually we divide our sprayed insects into four categories (1) *N*, not affected; (2) *S*, slightly affected; *i.e.* just able to walk when placed in an upright position; (3) *M*, moribund, *i.e.* insects able to move appendages but not able to walk when gently stimulated; (4) *D*, apparently dead. In the first two series of tests (samples Nos. 2 and 5, 5 and 6) the latter three categories were taken in assessing the effect of the poisons, but comparisons were also made on the basis of the sum of the percentages of moribund and dead insects. For subsequent tests we introduced a category intermediate between (2) and (3) to include insects badly affected but not actually moribund. The insects in this class could only move with extreme difficulty when stimulated with a camel-hair brush. After another 24 hours they were usually either moribund or dead. After the first two series of sprayings the percentage numbers of insects falling into the categories of badly affected, moribund and dead (*B*, *M* and *D*) were taken together as a means of judging the effect, but the percentage numbers of moribund and dead insects only are also given.

In Tables V, VI and VII the data for the six series of toxicity trials are given. The concentrations tested are expressed in terms of the various chemical factors determined, *i.e.* as mg. per 1000 c.c. of rotenone, ether

extract, methoxyl content, etc. For each concentration, the percentage of paralysed insects, with the standard error, are given, together with the corresponding probit (Bliss(1)). The control figures have been taken into account in expressing the percentages of insects affected by paralysis.

We have found Bliss's method of considerable value in reducing our data to a form suitable for statistical analysis, and although we have given one series (Fig. 1) in which the concentrations in terms of crude rotenone are plotted directly against the mortalities, we have found that

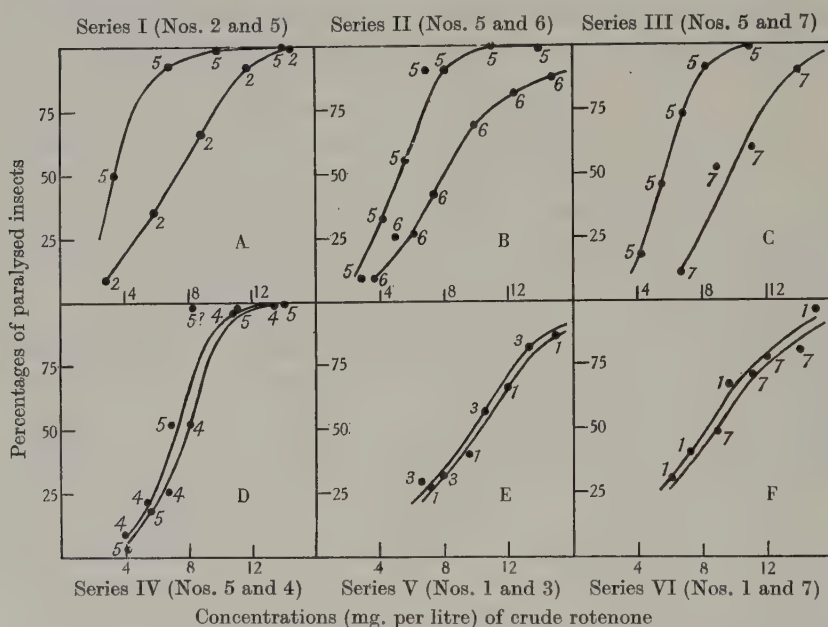


Fig. 1. Concentrations of crude rotenone plotted against percentages of paralysed insects for pairs of *Derris* samples.

by plotting the probits against the logarithms of the concentrations used, a clearer conception of the degree of concordance between our pairs of comparative tests is obtained. In all the figures except Fig. 1, therefore, the data are plotted in this way.

All the affected insects are taken into account in assessing the effect in Fig. 1 (sections A and B), and all the seriously affected insects in the remainder (sections C, D, E, F). In Fig. 2 these curves are converted to a probit log-concentration basis, and the freehand regression lines shown. Our comparisons are based upon the consideration that if any chemical method evaluates two samples correctly, then the regression lines relating

the effects produced to the concentrations should be coincident in position. If they are not coincident, the line to the left represents a sample which is more toxic with respect to the other than the determination in question would lead one to expect, *i.e.* this sample is undervalued by the determination with respect to the second. In several of the pairs of comparisons shown in Fig. 2 (A, B, C) which deal with crude rotenone, it is obvious that there are significant departures of the lines from coincidence and in no case is there an absolute concordance.

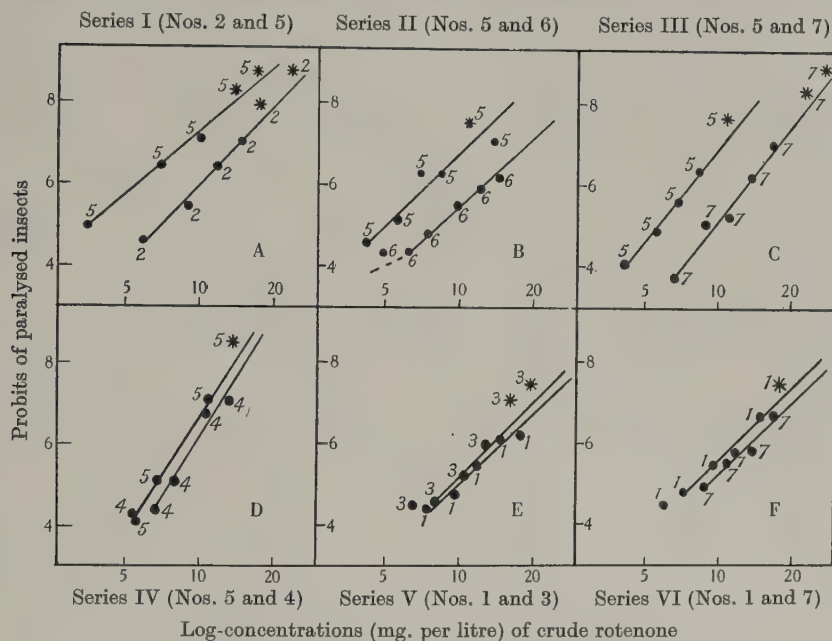


Fig. 2. Log-concentrations of crude rotenone plotted against probits of paralysed insects for pairs of *Derris* samples.

It is clear, therefore, that the determinations of crude rotenone do not give correct measures of the relative activities of all the pairs of samples.

In each of the remainder of the figures (Figs. 3-9) a separate pair of samples is compared. Each of the sections A-F expresses for the particular samples under consideration the relationship between the probit values and the log-concentrations, expressed in terms of the various chemical factors determined. We have thus on each chart a measure of the accuracy with which these six methods of analysis will express the comparative potencies of each pair of samples tested.

Analysis of results.

In Table V, series 1, and in Figs. 3 and 4 the data for samples Nos. 2 and 5 are given in tabular and graphical form.

In Fig. 3 the biological evaluation is based upon the probit values corresponding to the percentage numbers of moribund and dead insects, and in Fig. 4 upon the probits of the percentage numbers of insects showing paralysis. The two methods of assessing the effect enable one to draw similar deductions as to the relative values of the various

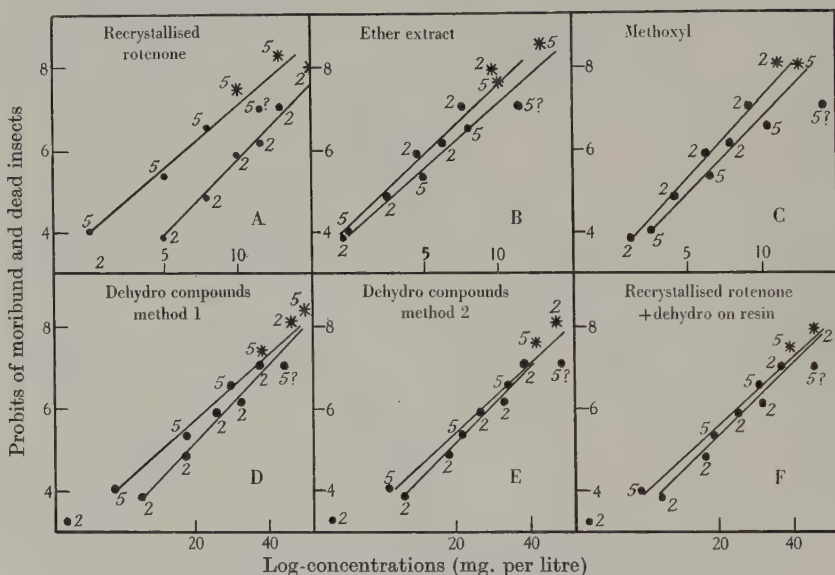


Fig. 3. Log-concentrations in terms of different chemical values plotted against probits of moribund and dead insects (series I, Nos. 2 and 5).

chemical determinations in indicating the order of activity of these two samples. Sample No. 2 (*Derris elliptica*) is relatively low both in recrystallised rotenone (1.41 per cent.) and in sodium-dried ether extract (6.8 per cent.). On the other hand No. 5 (*D. malaccensis*) is low in recrystallised rotenone (1.83 per cent.) but relatively high in ether extract (18.6 per cent.). We see from Figs. 3 and 4 that No. 5 is more toxic compared with 2 than the recrystallised rotenone content would lead us to expect, whether the evaluations are based upon all the paralysed or only upon the moribund and dead insects. Taking all the paralysed insects (Fig. 4) the ether extracts would, for this pair of samples, give a

TABLE V.

Comparisons of the toxicities of different samples of Derris root No. 2 (W. 148) with No. 5 (W. 151) and No. 5 (W. 151) with No. 6 (W. 150). Toxicity tests July 11 and 20, 1934. Insect used, *Aphis rumicis*. Fivefold replication, sprayed 10 insects at a time. Results 2 days after spraying.

S=slightly affected, M=moribund, D=apparently dead.

Concentrations (mg./1000 c.c.) tested, in terms of

No.	Derris samples	Root	Rotenone (crude)	Rotenone (recrys- tallised)	Ether extract	MeO	Dehydro compounds		Rotenone +dehydro compounds in residual resin	Insects* affected		Toxicity results				
							Takei separation, rotenone not separated	Separation from alcohol method 2		S + M + D %	Probits	S.E. %	M + D %	Probits	S.E. %	
SERIES I.																
1	No. 2 (W. 148).	1418	23.4	20	96	12	49.1	50.1	47.9	100	—	100	100	—	7.0537	+
2	<i>D. elliptica</i> , tuba	1063	17.5	15	72	9	36.8	37.6	35.9	100	± 2.0	98	98	± 2.0	6.1311	+
3	<i>D. elliptica</i> , tuba	886.5	14.6	12.5	60	7.5	30.7	31.3	30.0	97.9	± 4.8	87.2	87.2	± 4.8	5.9002	+
4	puteh, 28 months	709	11.7	10	48	6	24.5	25.0	24.0	66	± 5.8	81.6	81.6	± 5.8	4.4890	+
5		532	8.8	7.5	36	4.5	18.4	18.8	18.0	66	± 3.7	81.6	81.6	± 3.7	5.4125	+
6		354.5	5.85	5	24	3.0	12.3	12.5	12.0	34.7	± 14.4	12.2	12.2	± 14.4	3.8350	+
7		177	2.9	2.5	12	1.5	6.1	6.3	6.0	8.1	± 4.8	4.1	4.1	± 4.0	3.2493	+
8	No. 5 (W. 151).	1092	28	20	200	28.8	74.9	86.3	78.0	100	—	100	100	—	7.0537	+
9	<i>D. malaccensis</i> ,	820	21	15	150	21.6	56.2	64.8	58.5	100	—	98	98	± 2.0	7.0537	+
10	Saravak erect,	683	17	12.5	127	18	46.8	54.0	48.8	100	—	98	98	± 2.0	6.5464	+
11	28 months	546	14	10	100	14.4	37.4	43.2	39.0	100	—	98.9	98.9	± 2.5	5.3585	+
12		410	10	7.5	76	10.8	28.1	32.4	29.5	98	± 2.0	16.3	16.3	± 3.7	4.0178	+
13		273	6.9	5	50	7.2	18.7	21.6	19.3	92	± 5.8	6.2	6.2	± 3.7	—	—
14		136	3.5	2.5	25	3.6	9.4	10.8	9.8	49	± 10.9	6.2	6.2	± 2.0	—	—
15	Control (saponin solution + alcohol)	—	—	—	—	—	—	—	—	(2.0)	(± 2.0)	(2.0)	(2.0)	(± 2.0)	—	—
SERIES II.																
1	No. 5 (W. 151).	546	14	10	100	14.4	37.4	43.2	39.0	97.9	± 2.0	97.9	97.9	± 2.0	7.0335	+
2	<i>D. malaccensis</i> ,	437	11	8	80	11.5	30.0	34.5	31.2	100	—	93.6	93.6	± 2.45	6.5220	+
3	Saravak erect,	328	8.3	6	60	8.6	22.5	25.9	23.4	89.6	± 3.2	45.8	45.8	± 7.34	4.8945	+
4	28 months	273	6.9	5	50	7.2	18.7	21.6	19.5	89.6	± 5.8	29.7	29.7	± 5.13	4.7879	+
5		219	5.6	4	40	5.8	15.0	17.3	15.6	55.3	± 6.8	18.7	18.7	± 8.6	4.4870	+
6		164	4.2	3	30	4.3	11.2	13.0	11.7	33.3	± 7.3	6.2	6.2	± 5.8	4.1110	+
7		109	2.8	2	20	2.9	7.5	8.6	7.8	8.3	± 2.0	2.1	2.1	± 2.4	2.9665	+
8	No. 6 (W. 150).	322	14.8	12	37	5.3	21.7	22.3	22.7	87.3	± 7.3	61.6	61.6	± 9.7	5.2950	+
9	<i>D. polyantha</i> ,	268	12.4	10	31	4.4	18.1	18.6	18.9	81.2	± 8.0	5.853	5.853	± 5.1	5.3186	+
10	48 months	214	9.9	8	25	3.5	14.5	14.8	15.1	68.1	± 7.7	5.4705	5.4705	± 10.15	4.5295	+
11		161	7.4	6	18.5	2.65	10.9	11.1	11.4	41.6	± 3.03	4.7879	20.8	± 12.1	3.4186	+
12		134	6.2	5	15.5	2.2	9.0	9.3	9.5	25.6	± 5.7	4.3443	8.6	± 4.9	3.6342	+
13		107	4.9	4	12.5	1.77	7.2	7.4	7.6	24.9	± 7.3	4.3224	10.3	± 4.0	3.7354	+
14		80	3.7	3	9	1.33	5.4	5.6	5.7	8.3	± 4.9	3.6148	6.2	± 5.5	3.4618	+
15	Control (saponin solution + alcohol)	53	2.5	2	6	0.88	3.6	3.7	3.8	—	(± 1.8)	—	(4)	(± 1.8)	—	—

* Percentage allowing for control.

The standard errors were calculated on percentages before allowing for control.

good evaluation of the comparative toxicities. When, however, only moribund and dead insects are considered (Fig. 3) sample No. 2 appears somewhat more toxic than its ether extract would lead us to expect. The methoxyl contents (No. 2, 0.85 per cent.; No. 5, 2.64 per cent.) would also tend to undervalue No. 2 with respect to No. 5.

The concentrations of the dehydro compounds are expressed in three different ways based on (1) the yield given by Takei's method without the separation of rotenone, (2) a slightly modified Takei method (p. 587), (3) the determination of rotenone followed by that of the dehydro com-

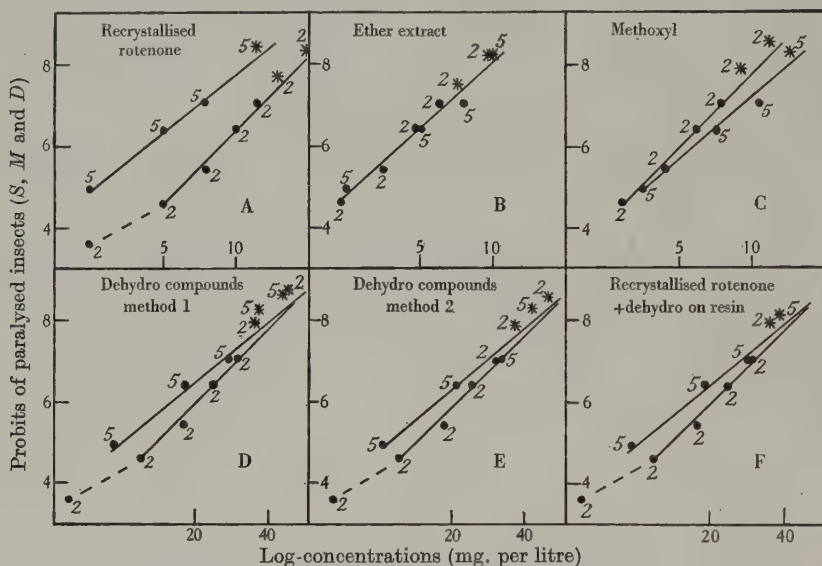


Fig. 4. Log-concentrations in terms of different chemical values plotted against probits of paralysed insects (series I, Nos. 2 and 5).

pounds in the residual resin. When the logs of the concentrations, expressed in each of these terms, are plotted against the probits of the percentages of all the paralysed insects, it is seen that these determinations had a tendency slightly to undervalue No. 5 as compared with No. 2. The two lines differ significantly from coincidence, although the discrepancy appears to be comparatively small. An analysis of the data Section E, Fig. 4, gave $\chi^2_b = 1.64$, indicating that the departure of the lines from parallelism is not significant, but $\chi^2_a = 10.61$, showing that they differ significantly in position (χ^2 for significance = 3.841 when $n = 1$ and $P = 0.05$). When only moribund and dead insects are taken, the estimation of the dehydro compounds still slightly undervalues No. 5,

but an analysis of the data of Fig. 3, section E, shows that the lines are not significantly different in position ($\chi_b^2=2.22$, $\chi_a^2=1.68$).

In Table V, series II, the data are given for the comparisons between samples No. 5, *D. malaccensis* (recrystallised rotenone 1.83 per cent., ether extract, 18.6 per cent., methoxyl 2.64 per cent.) and No. 6, *D. polyantha* (recrystallised rotenone 3.73 per cent., ether extract 11.5 per cent., methoxyl 1.65 per cent.). The probits of the percentages of all the paralysed insects are plotted against logs of the concentrations in Fig. 5.¹ The determination of recrystallised rotenone, ether extract or methoxyl

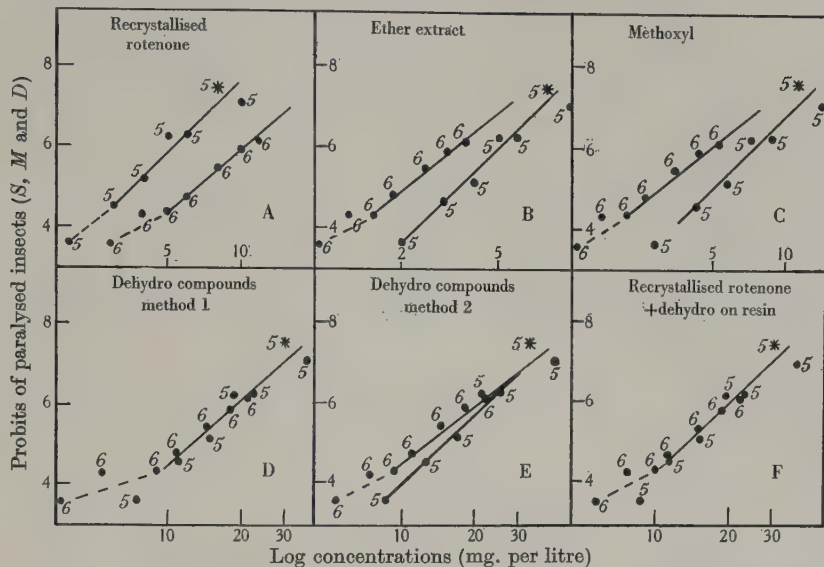


Fig. 5. Log concentrations in terms of different chemical values plotted against probits of paralysed insects (series II, Nos. 5 and 6).

content have not given accurate estimates of the comparative insecticidal values of these two samples. No. 5 is more toxic than the rotenone estimation would lead us to expect when compared with No. 6, while the ether extract and methoxyl content have undervalued No. 6 compared with No. 5. The dehydro determinations give for these two samples a more accurate comparative evaluation, the regression lines in D and F being scarcely different from coincidence. For method 2 (section E) while $\chi_b^2=2.16$, $\chi_a^2 (=8.59)$ shows a significant departure from coincidence.

¹ The probits for the percentages of moribund and dead insects gave comparisons of the same order and led to similar conclusions, but the points for both samples had a greater scatter about their regression lines.

TABLE VI.

Comparison of the toxicities of different samples of Derris root No. 5 (W. 151) with No. 7 (W. 153) and No. 5 (W. 151) with No. 4 (W. 147). Toxicity tests 30th July and 15th August, 1934. Insect used, Aphis rumicis. Fivefold replication, 10 insects at a time. Results 2 days after spraying.

B = badly affected, *M* = moribund, *D* = apparently dead.

[illegible]

The $B+M+D$ and the $M+D$ percentages are given allowing for control figures of 5 and 4 % respectively. Means of results of tests Nos. 13 and 14 (figures in brackets). The standard errors were calculated on the percentages before allowing for controls.

* Probits for 100 % were determined from the freehand regression line. They are shown in figures marked by an asterisk.

The results of the comparative tests for Nos. 7 and 5 are given in Table VI, and the probit values are plotted against the log-concentrations in Fig. 6. We decided at this stage that the biological effect was best determined by taking into account the seriously paralysed insects, *i.e.* the badly affected, moribund and dead. The control figures were not so good as in the previous tests. Sample No. 7, probably *D. elliptica* (recrystallised rotenone 8.51 per cent., ether extract 24.0 per cent., methoxyl 3.5 per cent.) is richer in both rotenone and ether extractives than No. 5, *D. malaccensis*. The proportion of rotenone to ether extract is greater in No. 7

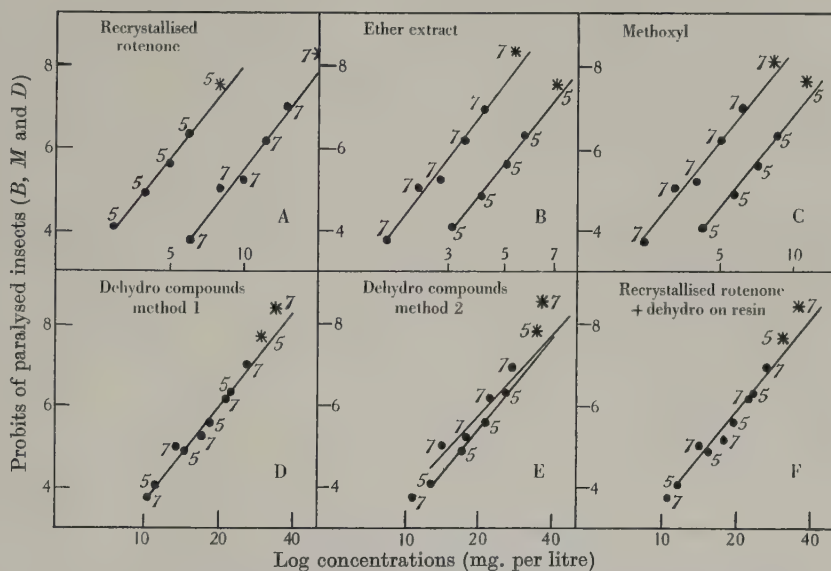


Fig. 6. Log concentrations in terms of different chemical values plotted against probits of badly paralysed insects (series III, Nos. 5 and 7).

than in No. 5. Although No. 7 is a better sample than No. 5, toxicity tests show that the difference in quality between these two samples is not fairly represented by the differences in rotenone content. In comparison with No. 7, sample No. 5 is undervalued by the rotenone determination and overvalued by the ether extract and methoxyl content. The estimation of the dehydro compounds, on the other hand, gives a closer measure of their relative potencies. The use of methods 1 and 3 gives points for the two samples which in each case can be adequately fitted by a single line. With method 2, however, there is a slight but significant departure of the lines from coincidence ($\chi^2 = 1.25$, $\chi^2 = 6.53$).

The data for samples Nos. 4 and 5 are given in Table VI, series IV. Both specimens are *D. malaccensis* var. Sarawak erect, the roots of No. 5 being 6 months older than those of No. 4. The recrystallised rotenone content of both are of the same order (No. 4, 2.0 per cent.; and No. 5, 1.83 per cent.). The percentages of sodium-dried ether extract and of methoxyl content are both slightly lower in the case of No. 4 than of No. 5 (15.4 and 2.16 per cent. against 18.6 and 2.64 per cent. respectively). In Fig. 7 the biological results are plotted in the way outlined (p. 597). It is seen that the percentage of rotenone slightly undervalues No. 5

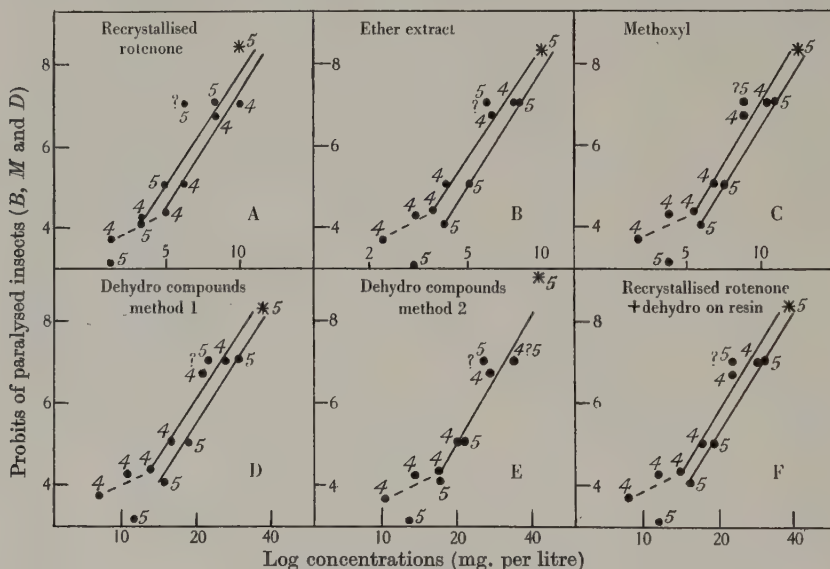


Fig. 7. Log concentrations in terms of different chemical values plotted against probits of badly paralysed insects (series IV, Nos. 5 and 4).

with respect to No. 4, and that the ether extract figure and the methoxyl content slightly undervalue No. 4 with respect to No. 5. The estimations of the dehydro compounds by methods (1) and (3) also undervalue No. 4 by comparison with No. 5, the difference between the regression lines being significant. For method 3 (section F) $\chi_b^2 = 0.14$, $\chi_a^2 = 12.73$. In the case of method 2 (section E), the data for both samples can be satisfactorily fitted by one regression line ($\chi_b^2 = 0.19$ and $\chi_a^2 = 3.49$).¹ In this comparison of two samples of *D. malaccensis* var. Sarawak erect, the determination of either rotenone, ether extract, methoxyl content or

¹ In the analysis of the data, test No. 9 (series IV, Table VI) was discarded, as the insects used were unsatisfactory and its incorporation introduced heterogeneity into the data.

of the dehydro compounds would not have given seriously erroneous evaluations of their relative activities.

In Table VII, series V, are set out the data obtained in the comparison of samples of *D. elliptica*, No. 3, Sarawak creeping, and No. 1, also *D. elliptica*, var. tuba puteh. The recrystallised rotenone contents are 3.83 and 2.80 per cent., the ether extracts 17.2 and 10.1 per cent., and the methoxyl contents 2.58 and 1.43 per cent. respectively. They have thus marked chemical differences. The data are graphed in the usual way in

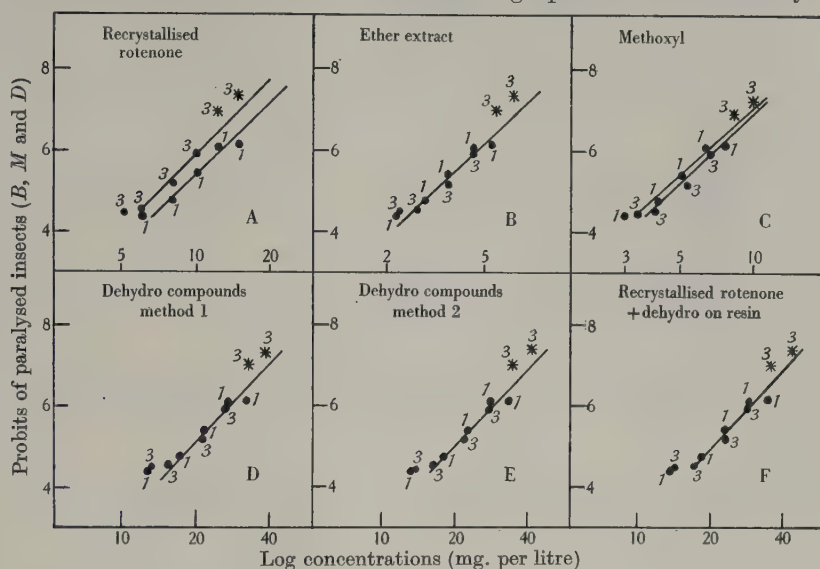


Fig. 8. Log concentrations in terms of different chemical values plotted against probits of badly paralysed insects (series V, Nos. 1 and 3).

Fig. 8. It is seen that with a possible exception in the case of the recrystallised rotenone, there is a close concordance between the various chemical determinations and the insecticidal activities shown by these two samples. An analysis of the data plotted in section E showed that there was no significant departure from parallelism or coincidence ($\chi^2_6 = 1.8$ and $\chi^2_a = 0.19$).

In Fig. 9, for samples Nos. 1 and 7 the probits of the paralysed insects are plotted against the logarithms of the concentrations expressed in terms of the different values estimated. In comparing the activities of Nos. 1 and 7 (Table VII, series VI), there was an abnormally high mortality in the control tests. We consider that the mean values given by the concentrations 7 and 12 may fairly be taken for purposes of estimating the net paralytic effect. It may, however, be observed that if

TABLE VII.

Comparison of the toxicities of different samples of *Derris root* No. 3 (W. 149) with No. 1 (W. 146) and No. 1 (W. 146) with No. 7 (W. 153). Tests made 21st and 8th August, 1934. Insect used, *Aphis rumicis*. Fivefold replication, 10 insects at a time. Results 2 days after spraying.

B = badly affected, M = moribund, D = apparently dead.

Concentrations (mg./1000 c.c.) tested, in terms of															
No.	<i>Derris</i> samples	Root	Dehydro compounds												
			Rotenone (crude)	Rotenone (recrys- tallised)	Ether extract	MeO	Dehydro compounds		Toxicity results						
							Takai separation rotenone not separated	Separation from alcohol method 2	Insects paralysed $B+M+D$ %	s.e. %	Probits of $B+M+D$	Insects $M+D$ %	s.e. %	Probits of $M+D$	
SERIES V.															
1	No. 3 (W. 149),	391.5	19.9	15	67	10	39.9	42.1	44.0	100	—	—	95.6	±4.0	6.7060
2	<i>D. elliptica</i> ,	326	16.6	12.5	56	8.4	33.2	35.1	36.7	100	—	—	95.5	±4.0	6.6954
3	Sarawak creeping,	261	13.3	10	45	6.7	26.6	28.1	29.3	81.8	±5.1	5.9078	58.2	±5.8	5.2070
4	22 months	209	10.6	8	36	5.4	21.3	22.5	23.5	56.8	±5.8	5.1713	36.3	±6.6	4.6495
5		156.5	8	6	27	4.0	15.9	16.8	17.6	31.8	±3.1	4.5267	14.3	±5.8	3.9331
6		130	6.6	5	22.5	3.4	13.3	14.0	14.7	29.5	±2.0	4.4612	20.9	±4.9	4.1901
7		104	5.3	4	18	2.7	10.7	11.2	11.7	(12)	(±3.7)	—	(8)	(±2.0)	—
8	No. 1 (W. 146),	536	18	15	54	7.7	32.8	34.3	35.4	86.9	±2.1	6.1217	78.9	±3.2	5.8030
9	<i>D. elliptica</i> ,	446	15	12.5	45	6.4	27.3	28.6	29.5	86.4	±3.7	6.0985	67	±3.8	5.4399
10	tuba puteh,	357	12	10	36	5.1	21.9	22.9	23.6	65.9	±0	5.4097	42.9	±3.7	4.8211
11	22 months	286	9.6	8	29	4.1	17.5	18.3	18.9	39.8	±3.7	4.7415	26	±1.9	4.3567
12		214	7.2	6	22	3.0	13.1	13.7	14.2	27.3	±7.5	4.3962	20.9	±5.6	4.1901
13		179	6.0	5	18	2.5	10.9	11.4	11.8	(11.7)	(±3.8)	—	(7.8)	(±2.6)	—
14	Control 0.5 gm./ 100 c.c. saponin +5 c.c./100 c.c. alcohol	—	—	—	—	—	—	—	—	(12)	(±3.7)	—	(12)	(±3.7)	—

The $B+M+D$ and the $M+D$ percentages are given allowing for the control figures of 12 and 9 % respectively, means of results of tests Nos. 7, 13, 14 (figures in brackets).

1	No. 1 (W. 146),	536	18	15	54	7.7	32.8	34.3	28.6	29.5	35.4	100 (100)	—	—	—	92.9 (94)	6.4684
2	<i>D. elliptica</i> ,	446	15	12.5	45	6.4	27.3	28.6	23.6	23.6	29.5	95.2 (96)	±2.4	6.6646	83.3 (86)	±2.4	6.4684
3	tuba puteh,	357	12	10	36	5.1	21.9	22.9	18.3	18.9	23.6	76.4 (80.4)	±6.9	5.7192	60.4 (66.7)	±6.0	5.9661
4	22 months	286	9.6	8	29	4.1	17.5	18.3	13.7	14.2	18.9	66.3 (72)	±8.0	5.4207	47.6 (56)	±5.4	4.9396
5		214	7.2	6	22	3.0	13.1	13.7	11.4	11.8	14.2	39.8 (50)	±5.5	4.7415	19 (32)	±5.8	4.1221
6		179	6.0	5	18	2.5	10.9	11.4	9.2	9.4	11.8	30.1 (42)	±5.8	4.4785	19 (32)	±3.7	4.1221
7		143	4.8	4	14.5	2.0	8.8	9.2	27.2	26.8	9.4	(16)	±6.0	—	(16)	±6.0	—
8	No. 7 (W. 153)	176	17	15	42	6.2	25.9	27.2	22.6	22.4	26.8	95.2 (96)	±2.4	6.6646	90.5 (92)	±3.7	6.3106
9	tuba root from	147	14	12.5	35	5.1	21.6	22.6	18.1	18.0	22.4	78.8 (82.4)	±4.6	5.7995	51 (58.8)	±8.0	5.0251
10	Paya Lebar, Sin-	118	11.1	10	28	4.1	17.3	18.1	14.5	14.3	18.0	71.0 (76)	±5.0	5.5534	35.7 (46)	±10.3	4.6335
11	gapore, <i>D. elliptica</i> ,	94	8.9	8	22.6	3.3	13.8	14.5	10.9	10.7	14.3	47 (56)	±7.5	4.9247	26.2 (38)	±7.35	4.3628
12	(probably)	70	6.7	6	17	2.5	10.4	10.9	—	—	10.7	1.2 (18)	±6.0	—	(16)	±6.8	—

In series VI the control tests were of such doubtful value that the means of tests Nos. 7 and 12 have been taken for calculating the $B+M+D$ and $M+D$ percentages (16 and 17) respectively. They may be slightly too high.

The $B+M+D$ and $M+D$ percentages in which these controls were not allowed for are given in brackets.

The standard errors are calculated on the percentages before allowing for controls.

The probit figures for 100 % were determined from the freehand regression line. They are shown in the figures marked by an asterisk.

no allowance is made the general conclusions that may be drawn are very little affected. They are very similar to those drawn from the preceding test. When the rotenone contents are taken as a basis of comparison, the regression lines show a slight difference in position, but in the case of the ether extracts, methoxyl contents and of the dehydro compounds, one line is seen to fit each set of data (*e.g.* section E, $\chi_b^2=0.03$, $\chi_a^2=0.11$). We have good reason to believe that both samples 1 and 7 are *D. elliptica*. This similarity in species may account for the closeness with which the various methods of analysis have given the comparative values of these two roots.

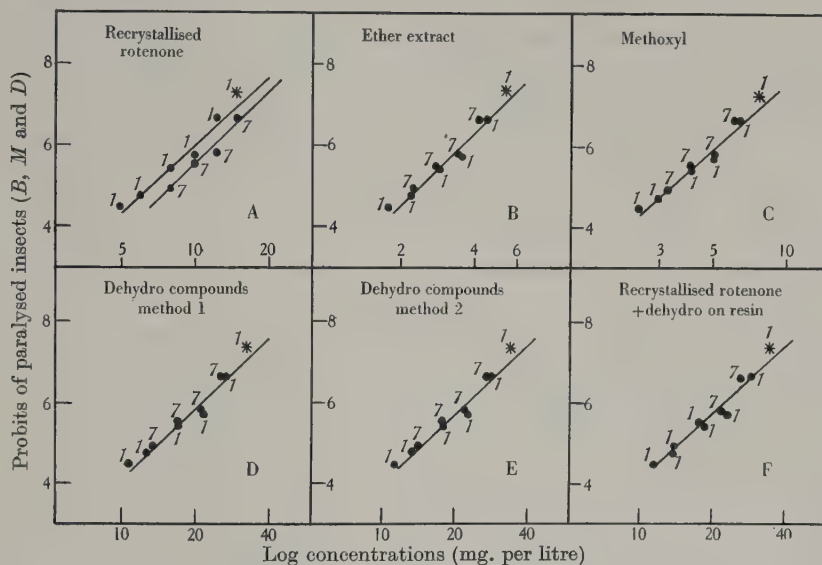


Fig. 9. Log concentrations in terms of different chemical values plotted against probits of badly paralysed insects (series VI, Nos. 1 and 7).

While this paper was in the course of preparation an important communication by Cahn and Boam (2) gave a new technique for determining crude rotenone, and for purifying it by triturating with alcohol saturated with rotenone. Trichlorethylene is used for the process of extraction and after its evaporation carbon tetrachloride saturated with rotenone is employed for the isolation of the rotenone. We have preferred to carry out the crystallisation of the carbon tetrachloride complex and the subsequent trituration at 0° C. The results for crude rotenone are given in Table II (p. 583) and agree quite closely with the values we obtained by using ether as extraction solvent and carbon tetrachloride for isolating

the rotenone, except in the case of sample No. 7, in which the Cahn and Boam method gave lower results, but which agreed with those obtained for this sample when carbon tetrachloride was used for extracting and crystallising. The figures obtained for both crude and purified rotenone by this process are not sufficiently different from those plotted to affect the nature of the conclusions drawn.

DISCUSSION.

Seven samples of *Derris* root have been chemically analysed, and for each the percentage content of crude rotenone, recrystallised rotenone, ether extract, methoxyl content, dehydro compounds, and rotenone, together with the dehydro compounds in the residual resin, have been determined. Our experience indicates that considerable care is requisite in the process of sampling the roots in order to ensure that a representative sample is taken. In addition, we have noted that unless samples of root are very finely ground, there is a tendency towards segregation into rich and poor parts. Impalpable powders of *Derris* root can only be obtained with difficulty under laboratory conditions, rendering imperative a thorough mixing of the sample immediately before abstracting a portion for analysis. Moreover, it is almost certain that the methods for the determination of rotenone and of the dehydro compounds are not finally established, and it will no doubt be necessary to ensure that each method of analysis complies with a rigid standard technique.

The insecticidal potency of the seven samples has been determined, using *Aphis rumicis* as a test subject. Owing to the possibility of fluctuation in the mean resistance of our stock of insects, two samples of *Derris* root were tested at a time, and conclusions have been drawn only from comparisons of each pair. Our method of assessing the efficiency of a chemical method of analysis is based upon this comparison of pairs. Ideally, if any method of chemical analysis gave results precisely commensurate with the insecticidal potencies of *Derris* samples, the toxicity tests of these samples at the same concentrations in terms of the value determined should have the same effect, and the curves expressing the relationship of the concentrations used to the effect (or the logarithms of the concentrations to the probits) should be identical with each other. Such an ideal is not likely to be strictly attained. If, however, the insecticide tests are replicated a number of times with pairs of samples belonging to the same or different species and varieties, and the results of one of the methods of analysis consistently gives curves and the corresponding regression lines approximating to coincidence, one may

have confidence in the method as a means of evaluation. We have attempted in Table VIII to indicate the degree of reliance that could be placed upon various methods of analysis as a means of giving comparative evaluations of our samples. It cannot be said that the determination of rotenone, ether extract, or methoxyl content, could separately be relied upon to give correct evaluations of all samples of *Derris* root. In samples in which the proportion of rotenone to ether extract is high, the determination of the rotenone content by the present methods tends to give an exaggerated value of the potency of the root and, conversely, to underestimate the potency of samples where this ratio is low. In samples where the proportion of rotenone to ether extract is low, the determination of the ether extract will tend to overvalue the insecticidal activity. These generalisations may only be strictly true when comparisons are made between different species. For our samples of the *same* species of *Derris* we have found that the determination of rotenone or ether extract or methoxyl content would each give approximately correct comparative values. Our experiments further indicate that the determination of the total dehydro compounds, or of rotenone plus the dehydro compounds in the residual resin, may give a better but not entirely

TABLE VIII.

Comparison of insecticidal activities between pairs of samples of the same and of different species, when the concentrations tested are expressed on the basis of the figures obtained by different chemical determinations.

	Samples compared of same species			Samples compared of different species		
Concentrations expressed as	4. <i>D. malaccensis</i> , Sarawak erect; 5. <i>D. malaccensis</i> , Sarawak erect	1. <i>D. elliptica</i> , tuba puteh; 3. <i>D. elliptica</i> , Sarawak creeping	1. <i>D. elliptica</i> , tuba puteh; 7. <i>D. elliptica</i> , from Paya Lebar	5. <i>D. malaccensis</i> , Sarawak erect; 6. <i>D. polyantha</i> , (<i>D. elliptica</i> ?)	5. <i>D. malaccensis</i> , Sarawak erect; 2. <i>D. elliptica</i> , tuba puteh	5. <i>D. malaccensis</i> , Sarawak erect; 7. <i>D. elliptica</i> , from Paya Lebar
Crude rotenone	Slightly discrepant	Slightly discrepant	Slightly discrepant	Widely discrepant	Widely discrepant	Widely discrepant
Recrystallised rotenone	Slightly discrepant	Slightly discrepant	Slightly discrepant	Widely discrepant	Widely discrepant	Widely discrepant
Ether extract	Slightly discrepant	Concordant	Concordant	Widely discrepant	Slightly discrepant-concordant	Widely discrepant
Methoxyl	Slightly discrepant	Slightly discrepant	Concordant	Widely discrepant	Slightly discrepant	Widely discrepant
Dehydro compounds, method I	Slightly discrepant	Concordant	Concordant	Concordant	Slightly discrepant	Concordant
Dehydro compounds, method II	Concordant	Concordant	Concordant	Slightly discrepant	Slightly discrepant	Slightly discrepant
Rotenone + dehydro compounds on resin, method III	Slightly discrepant	Concordant	Concordant	Concordant	Slightly discrepant	Concordant

accurate assessment of the insecticidal potency of *Derris* root to *Aphis rumicis*. This is true for our comparisons not only between samples of the same species of *Derris* but also between samples of the two different species, *Derris elliptica* and *D. malaccensis*.

It will be seen from Tables I and II that the proportion of rotenone to total ether extract is lower for samples 4 and 5 (*D. malaccensis*) than it is for the remaining samples, all of which are probably *D. elliptica*. This lower proportion of rotenone to ether extract in samples of *D. malaccensis* has been referred to by Georgi and Teik (5).

It is interesting to note in the case of all our samples which we believe to be *D. elliptica*, the methoxyl content calculated on the dehydro compounds accounts for some 60–70 per cent. of the total methoxyl content of the roots, whereas, in the samples of *D. malaccensis* tested, the methoxyl derived from the dehydro compounds was of the order of 40–50 per cent. of the total amount present. It may be possible, by observations of this kind, to distinguish, on a chemical basis, between *D. elliptica* and *D. malaccensis*.

SUMMARY.

1. Seven samples of *Derris* root have been examined chemically, and the following determinations carried out: rotenone (crude and recrystallised), ether extract, methoxyl content, and dehydro compounds. The importance of using standard methods of analysis is stressed.

2. Insecticide tests have been carried out and comparisons made between pairs of samples tested on the same day.

3. When comparisons were made between pairs belonging to *different* species of *Derris*, the determinations of rotenone by the present methods, ether extract or methoxyl content did not express accurately the relative insecticidal potencies of the pairs of samples. When comparisons were made between pairs of the *same* species, all these determinations appeared to give a closer measure of their relative activities.

4. In our samples, the estimation of the dehydro compounds, or of rotenone plus the dehydro compounds in the resin, gave a better assessment of the relative potencies than the other determinations, whether comparisons were made between samples of the same, or of different species. Further work is, however, needed.

We desire to express our great indebtedness to Mr C. D. V. Georgi for duplicate analyses of our samples and for valuable criticisms and suggestions during the course of this work. We wish also to express

our thanks to Mr P. C. Bowes for help in the analytical work, and to Mr A. Ogglesby and Miss I. Randall for considerable assistance in the insecticide tests.

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REVIEWS

Toximetrische Bestimmung von Holzkonservierungsmitteln. By LIESE, NOWAK, PETERS and RABANUS. *Angew. Chem.* **48**, 21, 1935.

In the field of pest control one of the most difficult problems is to find methods by which the effectiveness of fungicidal or insecticidal treatments can be estimated correctly from laboratory tests. It is of interest, therefore, to find that a standard laboratory test has been evolved for determining the toxicity to fungi of antiseptics for the preservation of wood. At a conference of European workers under the chairmanship of Hermann von Schrenk held in Berlin in 1930 there was general agreement as to the nature of the test required, and a committee was then set up to organise a series of co-operative experiments on the results of which decision could be reached as to the details of the test.

The results of these experiments have just been published in a reprint which gives a list of the test fungi chosen and indicates which of the isolations tested should henceforth be used in standard tests. In one of the experiments reported a series of tests upon identical samples of two antiseptics was carried out in a number of laboratories to determine how closely the results would tally when the standard method was followed. A detailed description of the method which has been adopted for carrying out the recommended "wood-block" test (*Klötzchen Methode*) is given, and a technique for making tests in agar medium (*Röhrchen Methode*) is also described which, it may be noted, differs considerably from the method proposed in the U.S.A.

There is little doubt that more fundamentally reliable results can be obtained from the wood-block test than from an agar test in spite of the experimental difficulties of the former which lead to a certain variation in the actual figures obtained for the toxic points. It is very desirable that as far as possible in the future laboratory tests on the toxicity of wood preservatives should be carried out on the lines suggested in this carefully considered report so that results obtained in different laboratories shall be directly comparable. The authors of this report rightly stress the fact that toxicity though extremely important is only one of the factors which must be taken into consideration when assessing the practical value of any wood preservative.

W. P. K. FINDLAY.

The Diseases and Curing of Cacao. By H. R. BRITON-JONES. Pp. x+161, with 37 figs. London: Macmillan & Co. Ltd. 1934. Price 10s.

The author states that his "hand-book has been primarily prepared for Agricultural Officers and Planters" and therefore "Technical and detailed descriptions of the several parasitic organisms causing disease in Cacao have been deliberately avoided wherever possible", since "technicalities of this nature tend to obscure in their minds the main issue, namely, how to diagnose a specific disease from macroscopic symptoms under field conditions and how to control it". The pathological portion of the book contains three chapters dealing respectively with diseases of root, stem and pod with a fourth chapter devoted to "Witches' Broom Disease". Each disease is taken in turn, its geographical and host distributions are described, an account is given of the symptoms by which it may be recognised, and detailed practical suggestions are made for its control. In the case of root diseases the control measures, being more or less common to all, are dealt with together at the end of the chapter. The account of "Witches' Broom" seems perhaps a little overweighted by fifteen pages of verbatim quotation from the author's paper in *Tropical Agriculture*. The accounts of the diseases are in simple, clear language and illustrated by excellent

photographic reproductions of macroscopic symptoms. Here and there the author tends to forget his audience and gives mycological technicalities, exact spore measurements, etc., and throughout the book he seems to be uncertain whether to adopt English or decimal measures, in one sentence on p. 8 both systems occurring.

The author's point of view is that "The primary object on a cacao estate is not to cure disease but to produce cacao", that "Expensive recommendations are too often made for the control of a comparatively unimportant disease, without reference either to co-existing important diseases or even to far more important horticultural practices", and that "in the case of cacao the writer knows of no pathological circumstances under which spraying can be recommended until the monetary value of cacao as a marketable commodity is much higher than it is at present". Accordingly, throughout the volume, he emphasises control "by modifying agricultural practices in such a manner as to bring about conditions which are within the range of tolerance of the host and outside that of the parasite". This is a very sane point of view which could profitably be extended to many crops other than cacao.

The last chapter of the book deals with the preparation or "curing" of cacao and seems just a little out of place in a volume dealing primarily with cacao diseases. Still it is a useful account and brings together in a very practical way what is known on the subject. There are two bibliographies, one of cacao fermentation and another of cacao diseases and a good index containing two misprints on p. 160. The book is well written and produced and should fulfil its author's purpose.

WILLIAM B. BRIERLEY.

Diseases of the Banana (and of the Manila Hemp Plant). By C. W. WARDLAW. Pp. xii+615. London: Macmillan & Co. Ltd. 1935. Price 30s.

It may be said at once that Dr Wardlaw has produced a book of first class importance which, for many years, is likely to remain the standard work on diseases of the banana and manila hemp. It is in no sense a popular treatise but a reference monograph for technical plant pathologists containing detailed accounts of all the diseases of these plants which have been recognised and of the fungi, bacteria and viruses which cause them. Much of this knowledge is incomplete or controversial, and some of the contents of the volume will almost certainly not stand the test of time and further research, but it is a valuable thing to have such knowledge as we do possess collated and systematised by a worker who has had special experience of the problems. The author has made a critical survey of the entire literature of the subject and, in the light of his own research and experience, has stated the present position as fairly and accurately as would seem possible. In so doing he has earned the grateful thanks not only of plant pathologists but of all who have any concern with the growing and marketing of this important crop.

Following an interesting and all too short introductory chapter, the book is divided into four sections dealing respectively with soil-borne, vascular and stem diseases; plantation diseases of fruit and leaf diseases; virus diseases; and, finally, storage diseases. The first three chapters of Section I are devoted to banana wilt, better known, perhaps, as "Panama Disease" caused by *Fusarium cubense* E. F. Sm. The author and his colleagues at the Imperial College of Tropical Agriculture, Trinidad, have contributed materially to our knowledge of this disease, and the consideration of it here is, accordingly, very detailed. Dr Wardlaw himself is hopeful of the elimination of the disease and states that "the several important avenues opened up as a result of genetical and cytological investigations will undoubtedly lead ultimately to the production of the desired new immune variety". Chapter IV is a short one giving an account of the same disease in Manila hemp. Chapter V is devoted to "Bonnygate" disease caused by *Calostilbella calostilbe*. Chapter VI deals with stem, rhizome and root diseases caused by Basidiomycetes including the interesting "stone-making" fungus *Laccocephalum basilapoides* McA. and Tipp. Chapter VII describes

miscellaneous diseases of rhizomes and roots including nematode and "physiological" troubles. Chapter VIII is devoted to bacterial vascular diseases and Chapter IX to stem and heart rots. Section II begins with Chapter X, and this, and the following three chapters, contain straightforward accounts of various fungal and bacterial rots of fruit, whilst Chapter XIV is devoted to "physiological" disorders. In Chapter XV various leaf diseases are described and there are brief diagnoses of species of some thirty-three genera of other fungi associated with banana leaves. Chapter XVI, which commences Section III, is the longest in the book and contains a full account of "Bunchy Top" disease of bananas and its transmission by *Pentalonia nigronervosa*. In Chapter XVII a similarly named disease of Manila hemp is described which, the author notes, may be more closely allied to heart rot than to "Bunchy Top". Several other virus diseases of banana are known and are described in Chapter XVIII. The fact that the banana, a delicate fruit, has to be reaped whilst still immature, transported overseas under special conditions in refrigerated holds, and finally ripened at a higher temperature, means that the work of the pathologist is greatly increased since storage troubles must obviously be of immense importance. Section IV commences, therefore, with two useful chapters on the problems of transport and storage, and on the physiological and environmental factors which either influence or are directly responsible for fruit wastage. Chapters XXI-XXIV are then devoted to the various fungal and bacterial diseases which occur during transport and storage, and Chapter XXV to "physiological" diseases. In a final chapter the deterioration of the fibre of Manila hemp is considered.

There are four appendices. Appendix I is a nine-page list of species of bacteria and fungi, with synonyms, associated with the banana as saprophytes and parasites, and distinguished by the use of different type. Appendix II contains a pure cultural comparison of six strains of *Fusarium cubense* E. F. Sm. Appendix III contains some startling figures of banana production and the importance of this crop may be appreciated when it is realised that, in 1930, the United Kingdom imported nearly fifteen million bunches and the U.S.A. nearly sixty-three million, whilst the exports from Jamaica alone in that year amounted to nearly twenty-five million bunches. Appendix IV describes the temperature, humidity, carbon dioxide and storage conditions in the holds of banana boats.

The volume concludes with a bibliography of 560 citations which includes some 1934 publications, and a good index. The work is illustrated by two coloured plates, 292 text-figures, many of which are good reproductions of photographs which must have been taken under almost impossible conditions, and twenty-one tables. The book is pleasantly free from misprints but "Tetranychus" is wrongly spelled on p. 165, and, although "Ravenala" is correctly spelled three times, it is incorrect on p. 49 and in the index.

The author's treatment of the several diseases is unusually thorough, details being given, in each case, of history, geographical distribution and economic importance, symptoms, causal organism with mycological and pathological data, relevant ecological, agricultural, and general biological considerations and, finally, control and legislative measures. As with so many tropical plantation crops, spraying plays a very small part in disease control, the principal measures being cultural on a basis of eradication and exclusion. The book is written in plain straightforward language and, although over 600 pages are devoted to the diseases of one crop, there is no impression of padding. The book is, of course, largely a compilation, but it is not a mere compilation. Dr Wardlaw has himself carried out numerous investigations of disease in bananas and his intensive field experience of the problems and, throughout the book, one feels that insight and sureness of understanding which only comes from detailed first-hand knowledge of a subject. Even where collating other workers' results and views his critical acumen and sane judgment are very evident. It would be impossible in any notice of reasonable length to give an idea of the wealth of knowledge and experience the book contains, but, by producing a work of this calibre, Dr Wardlaw has placed himself in the very front rank of British plant pathologists, and his volume is something of a landmark in the science.

WILLIAM B. BRIERLEY.

Colloidal Electrolytes: a General Discussion held by the Faraday Society.
Pp. iv + 422. London: Gurney and Jackson. 1935. Price 18s. 6d.

This is the report of the proceedings of the Third Colloid Meeting organised by the Colloid Committee of the Faraday Society. The first meeting in 1930 discussed "Colloid Science Applied to Biology", and the proceedings were published early in 1931. The second meeting in 1932 discussed "The Colloid Aspects of Textile Materials and Related Topics", and the proceedings were issued at the end of the year. The third and last discussion was held in September 1934, and the proceedings appear as the present volume on "Colloidal Electrolytes". The three meetings were international in character and were successful in their aim "of securing an adequate forum in Great Britain for the presentation and discussion of material leading to advancement of Colloid Science". Taken together the three volumes form a most valuable cross-section of the field of colloid science in its more applied aspects, and the meetings themselves form a model of efficient organisation which biological societies might well follow.

The subject of colloidal electrolytes was discussed under the following heads: General: theory, methods and experimental technique; Special and Technical: soaps and other long-chain colloidal electrolytes, dyestuffs, silicates and silicic acid, proteins, other substances. The volume contains thirty-six scientific papers and the report of fifteen general discussions upon them. Much of the content has little immediate application in biology and is primarily of interest to physicists and physical chemists. On the other hand many biological phenomena are essentially based on colloidal mechanisms, and it is obvious that concepts and ideas from colloidal science must play a primary part in the interpretation of functional mechanisms in physiological action. Further, for example, in his daily use of biological stains the cytologist is operating on a colloidal basis, as is the microbiologist in his use of culture media, or the plant pathologist and entomologist in their use of spreaders in spraying techniques. Most of this biological work is carried out on a purely empirical plane with little or no knowledge of the colloidal principles underlying the routines employed. Undoubtedly many of these routines are capable of enormous improvement or replacement, and such progress will come most surely and rapidly from an understanding of the theory and principles underlying what is often the very crude application.

It is difficult to pick and choose the papers in this volume which seem of importance to biologists, but the following may perhaps be noted: "Properties of Solid Soaps", by Bowen and Thomas; "The Influence of Atmospheric Carbonic Acid upon the Surface Tension of Aqueous Solutions of Sodium Salts of Fatty Acids", by Lottermoser; "Some Industrial Applications of Colloidal Electrolytes", by Stewart and Bunbury; "Measurements of the Diffusion of Dyestuffs", by Valkó; "The Nature of the Aqueous Solutions of Dyes", by Robinson; "The Dyeing of Cellulose with Direct Dyestuffs", by Morton; "The Reaction between Protein Fibres and Substantive Dyestuffs", by Elöd; "The Proteins as Colloidal Electrolytes", by Jordan-Lloyd; "Some Electrochemical Properties of a Simple Protein", by Linderstrøm-Lang; "Interactions of Proteins and Nucleic Acid", by Caspersson *et al.*; "Colloidal Ions of Starch", by Samec; and "Very Fine Wood Fibres as Colloid Electrolytes", by Lottermoser.

The volume is not one which many biologists will need to purchase for themselves, but it is essentially one which should be available to biologists in libraries.

WILLIAM B. BRIERLEY.

Phytography as a Fine Art; comprising Linnean Description, Micrography and Penportraits. By Dr J. W. MOLL. Pp. xix + 534, with 7 plates.
Leyden, Holland: E. J. Brill. 1934. Price 15 Guilders.

In 1923 Prof. Moll and Dr H. H. Jansonius published an original and intriguing volume entitled *Botanical Pen-Portraits* which aimed at giving greater precision to the

description of botanical drugs and, therefore, greater accuracy in their identification. Their method seemed of obvious value in the description of drugs, timbers, feeding stuffs and other plant materials where the ordinarily accepted taxonomic criteria were largely unavailable. Even after a lapse of over ten years, however, the method has not come into extensive common use, probably because of the painstaking labour and meticulous accuracy involved. In the traffic of modern professionalised botany there is little time for the practice of such a jeweller's art as micrography although, as the author points out, "the use of well-defined technical terms is the very best means of economising the use of words in descriptions of all kinds", and even "a telegram style can very well show a certain modest beauty".

The present volume, although published quite independently, is really a sequel to the earlier one, and describes the application of the same method to plants. The work is arranged in five "books". Book I, entitled "Phytography in past, present and future", contains three short introductory chapters dealing with the development of phytography and the dependence of the art upon (a) a fixed, universal and rational terminology based on general morphology, and (b) the use of formal and rigid guiding schemes.

Book II, 320 pages, is the largest part of the volume and contains three divisions. The first, a "Synopsis of General Morphology", consists of about 2500 botanical terms classified, defined and described in some 750 numbered "Articles" and seven plates, and the author has tried to include every technical term in common morphological usage. As these are minutely systematised in a running organographical and anatomical scheme, commented upon, often in great detail and illustrated by a profusion of examples, these pages form a valuable work of reference. The author's range of knowledge is immense, and his commentary is not only informative but stimulating. Division II, forty-five pages, is entitled "Morphological Notes". In the previous synopsis the author restricted himself to the communication of facts and considerations essential to the definition of the technical terms, but he here elaborates his own ideas on certain fundamental concepts and controversial matters, *e.g.* analogy and homology, the nature and value of the individual, symmetry, orders of sequence, phyllotaxy and so forth. Division III, eighty pages, contains the author's "Guiding Schemes", organised in six groups and described as "good servants and bad masters". Group I contains nine schemes for general subjects such as symmetry characters, branch systems, grade of individuality, polymorphism etc.; Group II, fifteen schemes for general organography, including early development stages, determination of reproductive organs, sori and hymenia, organographical description of surface appendages, etc.; Group III, thirty-six schemes for special organography including the thallome, cormus, caulome, phyllome and root; Group IV, four schemes for cytology; Group V, fifteen schemes for general histology such as stomatal apparatus, internal glands, vascular tissues, etc.; Group VI, nine schemes for special histology such as highly differentiated thallomes and various flower structures, etc. It is quite impossible without extensive quotation to give any idea of the organisation and the minute detail of these guiding schemes which, so far as one can see, completely cover morphological needs. Plant descriptions in the exact terminology of Division I based on the schedules would certainly be more complete and standardised than anything I am familiar with in botanical literature.

Book III, twelve pages, refers to examples of the application of the method of Pen-portraiture in phytography and in certain other sciences. Of its application in zoology the author merely remarks "*Hodie desunt, cras abundabunt*". Book IV, twelve pages, relates the author's experience in the use of the phytographical method in his teaching. Book V, twenty-four pages, opens with an interesting historical chapter, passes to a consideration and criticism of botanical text-books and concludes with some rather tepid "Anticipations".

The meat of the volume is in Book II, and, although the remainder of the work is often of great interest and contains many quotable passages, it is all of rather secondary value and a little prone to moralising. Whether or not Prof. Moll's art can ever become the general basis of descriptive morphology seems to depend largely on the time factor. Were it adopted it might have surprising effects on morphology, although

I cannot help still thinking that accurate sketches or photographs are worth many words. Even the author states that "for many purposes pictures have become superfluous, but for full records they remain indispensable, because in many respects even the simplest plant shows essential, but unintelligible particulars". Further, "a complete portrait of a higher plant will easily take a man's work during a whole year" and "the completion of a work comprising the Pen-portraits of all known plants would cost at least 150,000 years of a man's work". Prof. Moll's "only alternative is that every botanist should be able to make a Pen-portrait when he wants it for his own special investigations", but, surely, most of us already do this, although not, perhaps, in so rigid a form as detailed in the author's guiding schemes.

Prof. Moll died in the autumn of 1933 when his work was in the press and the proof sheets were revised by Prof. Schoute, his successor at Groningen. There are several misprints and, occasionally, the grammatical form is a little awkward, but, on the whole, the book is written in good English and one must congratulate not only Prof. Schoute on his success on what can have been no easy task but also the publishers on producing a volume of such pleasant format.

In his preface the author describes how, at the age of 82, blind and increasingly deaf, he carried on his work to completion dependent entirely on his secretaries. "My ears could not replace my eyes, but my sense of touch could, and if Braille's splendid invention had not existed this book would never have been written". And yet, on p. 570, he writes: "This is a very difficult subject and of no special interest for our present purposes. I hope to return to it at some future occasion." Such courage is amazing, but then Prof. Moll was a great man. WILLIAM B. BRIERLEY.

Second Conference on Cotton Growing Problems; Report and Summary of Proceedings. Pp. 340. London: Empire Cotton Growing Corporation. 1934. Price 2s. 6d., post free.

Thirty-one papers presented by workers from the West Indies, Sudan, Nigeria, Kenya, Uganda, Rhodesia, Fiji, India and England are grouped under the headings: plant breeding and genetics, crop experimentation and statistical treatment, cotton pests, cotton diseases, botanical problems, fibre properties of cotton, and soil problems. As the papers were circulated some time before the meeting, the discussions, which are fully recorded, were more fruitful than is often the case. The *Report* is an admirable cross-section of modern research on the cotton plant and, as many of the ideas and problems discussed are by no means confined to cotton, it will be found suggestive by workers on other crop plants. Published at the remarkably low price of 2s. 6d. it should be in the hands of all scientific workers who have to deal in any way with this primary crop.

WILLIAM B. BRIERLEY.

Les Plantes Alimentaires chez tous les Peuples et a travers les Ages; Histoire, Utilisation, Culture. Vol. III. *Plantes a Épices, a Aromates, a Condiments.* By D. BOIS. Pp. 289, 71 figs. Paris: P. Lechevalier. 1934. Price 50 francs.

Vols. I and II by Prof. Bois, on vegetables and fruits respectively, are well known, since they are among the best compendia of their subjects. The present volume deals with plants and their products used in rendering food more appetising and palatable. The history of these plants is one of the more interesting chapters in the story of civilisation and, as the author says, "La recherche de leur provenance et leur commerce ont véritablement changé la face du monde". Prof. Bois opens with a short historical account which is particularly interesting as it contains a memoir of Pierre Poivre, a celebrated "voyageur", who lived from 1719 to 1786 and was largely responsible for the development of the spice trade between France and her colonies. The French word for pepper is "poivre", but of this the author says: "Le Poivrier et son fruit, le Poivre, contrairement à une croyance courante, ne tirent pas leur

noms de celui du botaniste qui s'est tant appliqué à propager les épices. Ils s'agit là d'une simple et bizarre coïncidence, car le Poivre a toujours porté cette appellation. Au XIII^e siècle Marco Polo, le célèbre voyageur vénitien, citait cette épice sous cette dénomination."

There is no logical order in the arrangement of the contents of the book which would, indeed, be almost impossible in a treatise devoted to this subject, since the qualities for which mankind values these plants and their products are *ad hoc* characteristics having little or no botanical significance. About one-half of the volume is devoted to spice plants including species of *Piper*, *Myristica*, *Ravensara*, *Monodora*, *Xylopia*, *Zanthoxylum*, *Eugenia*, *Pimenta*, *Cinnamomum*, *Canella*, *Illicium*, *Capsicum*, *Zingiber*, *Curcuma*, *Alpinia*, *Elettaria*, *Amomum*, *Aframomum*, *Crocus* and *Vanilla*. The author then passes to the seasoning and aromatic plant products such as mustard, horse radish, garlic, basil, peppermint, capers, etc., and to culinary oil plants such as the olive and less important sources. Plants yielding sugar are next described and include the maple, sugar cane, beet, etc., and finally, various minor plants possessing one or other culinary value.

The boundaries of the subject and its divisions are, of course, artificial and vague, but the author has covered his selected ground thoroughly. Certain plants are omitted which might, perhaps, have found mention, e.g. *Glycyrrhiza glabra*, the source of liquorice, and *Boswellia* spp. and *Balsamodendron* spp., which give respectively frankincense and myrrh, *Murraya Koenigii*, the leaves of which are used in curry powder, and so forth. The author gives full details of each species and often of its varieties, including the botanical characters of the plant and especially of the part yielding the culinary product, the cultivation and economic importance of the plant, the biochemistry of the product and, in most cases, interesting notes on the history of the plant in cultivation. The more important plants such as the economic species of the genera *Piper*, *Myristica*, *Eugenia*, *Cinnamomum*, *Capsicum*, *Zingiber*, *Crocus*, *Vanilla*, *Sinapis* and *Olea* are treated in considerable detail. A useful feature is that the common name of each particular product is given in any number up to twenty-six different languages and, as there is a splendid index which includes all these as well as the scientific names, it is very easy to track down any plant or product. On the other hand there is no table of contents, which is a distinct drawback, and there are very few references, which is a much more serious omission, since, in using such a book as this, one frequently wishes to go to more detailed and original sources of information. The work is illustrated by seventy-one line drawings in the text, which are indexed at the end, and there are occasional misprints. The book is No. VII of the *Encyclopédie Biologique*, the two previous volumes being Nos. I and III, and, as it is well up to the standard set by Prof. Bois in his earlier books it forms an extremely useful work of reference which all botanists will probably find occasion to consult.

WILLIAM B. BRIERLEY.

Die Hefesammlung des "Centraalbureau voor Schimmelcultures": Beiträge zu einer Monographie der Hefearten. I. Teil; Die sporogenen Hefen. By N. M. STELLING-DEKKER. Pp. vii+547. 1931. Price 10 F.
 II. Teil, 1. Hälfte; *Die anaskosporogenen Hefen.* By J. LODDER. Pp. xi+256. 1934. Price 7.50 F. Amsterdam: N. V. Noord-Holland-sche Uitgevers-Maatschappij.

All mycologists and plant pathologists recognise the importance of an organisation whence they can obtain "type" cultures of fungi and bacteria, and the "Centraalbureau voor Schimmelcultures" maintained in Holland has justifiably earned its high reputation. In most countries there is one or another source of pure culture supply, but there is no other institution in the world where the arduous and critical task of maintaining such a collection is done more adequately and on such a large scale as Prof. Westerdijk's institution at Baarn. The yeasts are a difficult and highly specialised

group of micro-fungi demanding exceptional methods of cultivation and study, and they are, therefore, treated as a distinct part of the collection. For this purpose the "Centraalbureau" at Baarn collaborates with the "Laboratorium voor Microbiologie der Technische Hoogeschool" at Delft which is under the distinguished direction of Prof. A. J. Kluyver, and it is here that the yeast cultures are maintained. The institutions at Baarn and Delft not only maintain and supply named pure cultures but they are also first class centres of research where physiological, pathological and systematic investigations are carried out upon the micro-organisms available. A noteworthy example of such research is the present treatise on the yeasts, a work which is essentially systematic and based on a critical examination of the Delft cultures.

In vol. I the sporogenous yeasts were reclassified on a more rational basis, the scheme of which was outlined in some detail, and the species described were referred to the following genera: *Saccharomyces*, *Endomyces*, *Monosporella*, *Saccharomycodes*, *Schizosaccharomyces*, *Menatospora*, *Ashbya*, *Zygosaccharomyces*, *Torulaspora*, *Pichia*, *Hansenula*, *Debaryomyces*, *Schwannomyces*, *Hanseniaspora*, *Nadsonia*, *Coccidiascus*, *Guilliermondella* and *Zygosaccharomycodes*.

In the recently issued first part of vol. II certain of the anascosporogenous forms are dealt with, namely, the *Rhodotorulaceae*, which contains the species with carotinoid pigments, and the subfamily *Torulopsoideae*, of the *Torulopsidaceae*, which contains the forms having no conidia or carotin and with a primitive or absent pseudomycelium. The second subfamily, the *Mycotoruloideae*, containing forms with a pseudomycelium and an "appareil sporifère", will be considered in the second part of vol. II. The classification of Langeron and Talice, which is a modification of that of Ciferri and Redaelli, is adopted and the species described are referred to the following genera *Rhodotorula*, *Torulopsis*, *Pityrosporium*, *Mycoderma*, *Kloeckera*, *Asporomyces*, *Trigonopsis*, and *Schizoblastosporion*. The retention of the genera *Eutorulopsis*, *Schizotorulopsis* and *Microblastosporon* is regarded as unjustifiable.

In Stelling-Dekker's work the original description and the results of the author's own examination were placed in parallel columns. In the present volume an account of the source of the particular culture of each species is followed by a summary of the original description. The author then gives the results of her own examination including cell dimensions and developmental stages after different periods of growth, characters and reactions in standard media and carbon and nitrogen relationships. Each description is accompanied by an original drawing of the particular organism and references to the literature, and each genus is followed by a species key while, at the end of the volume, there is a key for recognition of the seven genera of the subfamily *Torulopsoideae*. The volume opens with an admirable account of the history, biological and systematic relations of the Anascosporogenous yeasts and closes with good author and subject indices.

The amount of critical examination that has gone to the making of these volumes is immense, and yet the original work is so condensed and systematised that only a careful examination of matters with which one is familiar reveals it. The treatise, when completed, will rank as the standard work of reference for all questions of yeast taxonomy and nomenclature, and its production reflects great credit on the "Centraalbureau voor Schimmelcultures" and in particular on Mrs Stelling-Dekker and Miss Lodder who have performed so successfully their arduous task.

WILLIAM B. BRIERLEY.

List of Common Names of British Plant Diseases. Compiled by the Plant Pathology Committee of the British Mycological Society. Pp. 95. Cambridge: University Press. 1935. Price 2s. 6d.

The list of common names of British plant diseases or, more accurately, of diseases of agricultural and horticultural plants commonly grown in Great Britain, published by the British Mycological Society in its *Transactions*, 1929, has, by its usefulness,

already justified the great amount of labour expended by the Plant Pathology Committee on its production. In the intervening period various emendations have been suggested and new diseases recorded, and these are incorporated in the revised list. The present issue is a thoroughly practical piece of work which should be in the hands of all British plant pathologists and adopted by them as a working basis.

WILLIAM B. BRIERLEY.

Epidemics and Crowd Diseases. By MAJOR GREENWOOD, F.R.S. Pp. xii + 409, 74 tables, 8 graphs. London: Williams and Norgate. 1935. Price 21s.

Owing to the special factors of its development the study of plant diseases and pests has followed restricted lines. Our knowledge is derived from the labours of mycologists (more recently plant pathologists), entomologists, etc., and due, partly, to the fact that symptoms are often indefinite and comparatively unreliable as diagnostic criteria and, partly, to the fact that the primary interest of the investigators has been the fungi, bacteria, insects, nematodes, etc., involved as causal agents, we are in the position of knowing a great deal about pathogenes *qua* pathogenes but little about disease *qua* disease. Further, our knowledge of disease, such as it is, is knowledge of individual hosts and their reactions to invasion, that is, it is essentially clinical knowledge. In the case of plants which are cultivated as individuals the clinical attitude is, with certain limitations, adequate but, as most crop plants are cultivated as populations, the factor of disease epidemicity enters in. Concerning the epidemiology of plant diseases our knowledge is slight, and this is due, partly, to the reasons given above, but, much more, to the fact that epidemiology is essentially a statistical science, and the statistical methods basic in its study have been largely unavailable or, if available, largely outside the scope of the common run of us. In clinical study the unit is the individual, whereas in epidemiological study the unit is the population and, in experimental epidemiology, the environmentally controlled and statistically analysed population. In the case of certain animal and human crowd diseases a study of the epidemic factor has been commenced with, in many cases, somewhat unexpected results and there can be, I think, little doubt that if plant pathologists and entomologists could apply similar methodical principles of study to plant diseases and pest attacks, the results would be equally illuminating. A tentative beginning has been made in connection with cereal rusts and smuts, potato blight, etc., and in regional surveys of plant diseases and pests, but the "vital statistics" of crop diseases which are the real basis of a science of epidemiology are practically absent, as may be appreciated by comparing, for example, the *Annual Reports of the British Registrar-General* with publications recording plant-disease data. One of the primary needs of our science is "vital statistics", and their absence is holding up progress perhaps more than any other single factor. Plant diseases, more than human diseases or the crowd-diseases of rats and mice and guinea-pigs studied by Prof. Greenwood, are suitable material for the experimental study of epidemiology, and the applied biologist should play a major part in the development of this science.

The present book is a non-technical introduction to the study of the epidemiology of human diseases. It will not give to the biologist any statistical tools which he can apply directly in his own researches; for these he must go to technical books by statisticians: nor will it, of course, give him any data on plants, for these have not yet been collected. What the book will do is to stimulate his imagination and give him fresh ideas and novel points of view and, speaking as a plant pathologist, I feel strongly that, unless we get some fresh ideas and novel points of view, not only shall we make little headway in understanding the epidemiology of plant disease but much of our science will stagnate. In the present state of plant pathology one new idea is worth a thousand of the routines repeated in our laboratories and experimental fields.

Prof. Greenwood's book is divided into two parts, the first nine chapters dealing with the general principles of epidemiology and the more important methods of

investigation, and the remaining fifteen chapters applying these principles and methods to particular cases of crowd diseases among men. In the first four chapters the author introduces his subject historically—from Hippocrates to Galen, through the revival of learning to Graunt and on to Pasteur and Galton—and this with a view of showing that a real science of epidemiology could only come into being when statistical methods become adequate. Chapter v is a fascinating and all too brief account of experimental epidemiology, in the course of which the author says: "Epidemics behave so wilfully. The population will not stay put; all kinds of disturbing and—as we think—irrelevant factors destroy the simplicity and symmetry of the phenomenon. How much better it would be if we could mould a little epidemiological world of our own nearer to the heart's desire. That is the art of the experimental epidemiologists. He brings into existence a little world of his own, introduces into that world some sickness, and watches what happens." Better, perhaps, providing we always remember that we are dealing with a little moulded world of our own near to our heart's desire, and do not reason straightway from our artificial microcosm to the macrocosm. A chapter on the artificial immunisation of man links the previous topics with a consideration of predisposing or "Procataretic" factors in disease, which occupies the remaining three chapters of Part I.

Part II considers the epidemiological aspects of a number of important diseases: the typhoid group, cholera, typhus, measles, diphtheria, scarlet fever, smallpox, plague, epidemic diseases of the central nervous system, influenza, venereal diseases, tuberculosis and cancer. There are also interesting chapters on the "vaccination controversy". This part of Prof. Greenwood's book is full of matter not only interesting in itself but most suggestive to an applied biologist, since many of the epidemic phenomena of human disease are reminiscent of phenomena in plant disease. This may be analogy and coincidence, but it seems more likely to be the exemplification of common principles.

Occasionally in the text the naïveté of the statistician peeps out, as, for example, when the author says "most people nowadays are aware that the coefficient of correlation is not a satisfactory measure of the association of two variables when the law of increase of the mean value of one variable as the other increases (or decreases) is not approximately represented by a straight line". Speaking by and large, one would be very surprised if "most people" had ever heard of the coefficient of correlation. On the whole, however, the author's writing is unusually good, vigorous and full of charm, and most readers of the book will probably agree with Prof. Greenwood in believing that "this is a field of study not only as important but as interesting as others universally agreed to be within the circle of general culture. The subject is one which the non-professional reader has no excuse for neglecting on the ground that it is dry and technical. If and when all educated persons are as familiar with this kind of medical history as they are with political history, the level of discussion of social legislation will be raised."

WILLIAM B. BRIERLEY.

The Struggle for Existence. By G. F. GAUSE. Pp. ix+165. 39 figs. Baltimore: The Williams and Wilkins Co. (Agents, Baillière, Tindall & Cox, London.) 1934. Price 13s. 6d.

For three-quarters of a century "The Struggle for Existence" has been a household phrase in biology. Once the idea was formulated it seemed so obvious as hardly to need detailed experimental verification and, in consequence, its verification under exactly controlled experimental conditions was neglected. Yet, as Raymond Pearl says in his foreword: "If ever an idea cried and begged for experimental testing and development, surely it was this one." The last two decades have shown an altered mental attitude in biologists, and credentials are being demanded for much that was accepted somewhat blindly by ardent neo-Darwinians. Statisticians and geneticists are largely responsible for this, and one result has been that the dynamics of populations has become a rather favourite topic of study among ecologists and microbiologists. Dr Gause, who works in the Zoological Institute, Moscow, has been engaged for some

years in experimental and statistical research on the struggle for existence in various micro-organisms, more especially Protozoa and yeast, and here brings together in connected form results which have been published in various *Journals*.

The first half of the book is introductory, Chapter I containing an interesting statement of the theory of the problem in its more statistical aspects, Chapter II considering generally the struggle for existence among animals and plants in natural conditions, and Chapter III portraying the mathematical view of the problem. This part of the book is extremely interesting and, though most of the subject material is a little obvious, it is always valuable to have old material set out in a new way. The remaining three chapters contain the author's own work and deal respectively with the mechanism of competition between yeasts (*Saccharomyces cerevisiae* and *Schizosaccharomyces kephir*), the competition for common food among Protozoa (*Paramecium caudatum*, *P. aurelia* and *Stylonychia mytilus*), and the destruction of one protozoan by another (*P. caudatum* and *Didinium nasutum*). There are two short appendices containing tabular data and statistical method, a bibliography of 138 citations and an index. Throughout the book the name "Calkins" is wrongly spelled.

The author's ideas and statistical technique derive essentially from the work of Pearl, Lotka, and Volterra, and he does not seem to be equally familiar with the researches of English statisticians and biologists. His own investigations are of a type that has long been familiar to microbiologists and, in the literature of this subject, Dr Gause will find numerous data illustrating his thesis. The author's work is a somewhat preliminary attempt to give experimental proof of the struggle for existence among certain micro-organisms and to express in statistical form particular aspects of that struggle. The book is really a sketch for a much larger work.

WILLIAM B. BRIERLEY.

Coffee in 1931 and 1932: Economic and Technical Aspects. By W.

BALLY. Pp. 231. Rome: International Institute of Agriculture. 1934.

Price 20 Liras.

This is the first of a series of monographs on the principal crops grown in tropical countries, and the work is based partly on a survey of the relevant literature and partly on information received directly from government and other authorities in coffee-growing countries. An introductory chapter stresses the immediate and primary importance of financial, economic and political questions over and above all problems of agricultural improvement, diseases and pests, and other cultural aspects. Chapter II contains statistical data on production, consumption, and prices of coffee in 1931-2 as compared with previous years, and enables one to gain some idea of the factors which led to the insane position where millions of bags of coffee were destroyed at considerable expense while large populations could not obtain the beverage. In two and a half years nearly twelve million bags of coffee were destroyed in Brazil alone, and the author remarks that "an impartial observer who compares these figures can only conclude that the destruction actually carried out was insufficient to fulfil the purpose desired". Chapter III surveys the economic aspects of coffee growing in the various coffee-producing countries of the world, and, as Brazil produces 65 per cent. of the world's coffee, the situation in this country receives detailed consideration. The chapter ends with a discussion of the international aspects of coffee growing. Chapter IV is a literature review of the technical, plantation and scientific aspects of coffee growing and it is a fairly complete and useful summary of our present knowledge. Chapter V is devoted to diseases and pests of coffee. The author is himself a distinguished plant pathologist who in 1931 published a treatise on "Die ziekten van de koffie", and this chapter is based on his own book and work issued since its appearance. The final chapter deals with the product and its preparation.

In each chapter the particular topics are treated geographically by countries and, wherever possible, textual statements are illustrated by tabular data or graphs. Each chapter is followed by an extensive bibliography and these, together, very

completely cover the problems of coffee growing. There is a good table of contents but unfortunately no index. The volume is essentially a 1932 cross-section of the world coffee situation. Much of the literature on coffee is not only in Dutch or Spanish but is widely scattered in journals and books not easily available, even could one read them. In collating and summarising this work in well-written English Dr Bally has done useful service and his book will be valuable to all interested in the growing and marketing of coffee.

WILLIAM B. BRIERLEY.

Primitive Land Plants: also known as the Archegoniatae. By F. O. BOWER. Pp. xi+658. London: Macmillan & Co., Ltd. 1935. Price 35s.

In 1908 Prof. Bower published *The Origin of a Land Flora*, and, for many students of my generation, the work became a sort of botanical bible. For sheer mastery and logical systematisation of data in relation to a system of reasoning it still seems to me almost without equal in English botanical literature of this century. Since 1908 Prof. Bower has published many technical and popular works and now after nearly thirty years he returns to his first love, alas! to many younger eyes, perhaps a little faded. Although the theme of the present book resembles that of the *Land Flora* and the same general attitude towards alternation of generations, *i.e.* an interpolation of an advancing diploid sporophyte, is maintained, *Primitive Land Plants* is not a new edition of the earlier work. It is an overlapping and complementary volume which brings to a focal point the relevant morphological and organographic investigation of the interim period. This latter has been one of marked advance, and especially important perhaps is work on the Devonian Psilophytales which has revealed a series of synthetic forms bridging the old gap between the mosses and the ferns, Zimmerman's "Telome Theory" and Bower's own contribution to our realisation of the influence of "the inward urge towards increase of size, with its attendant elaboration of primary form and structure which is inherent in all vegetation".

The material of the book is divided into two parts. In the first twenty-three chapters the several classes of the Archegoniatae are described and compared, beginning with the primitively simple Anthocerotales and progressing to the more recent and complete Filicales. This part of the work is a statement of the premises on which the author builds his argument, and it shows the same wizardry in the handling and marshalling of data which, in the old *Land Flora*, so gripped our imagination and stimulated us as students. The following six chapters are each devoted to the discussion of some one feature common to all Archegoniates and contain the argument itself. In Chapter xxx the author consolidates his position and formulates a general organographic analysis of the Archegoniates and, in a final chapter, summarises his results and conclusions. The general aim of the work is to lay a foundation for the organographic treatment of land plants at large by intensive study of primitive forms. The more immediate aim is not to trace phyletic relations but to visualise the methods of advance which primitive land plants have followed in their evolution. The evidence on which the reasoning is based is derived from the living Archegoniates, and the reasoning itself is checked by reference to fossil forms.

Prof. Bower's work is an essay in pure botany and cannot in any sense be reviewed in these pages. It is the culmination of a lifetime's thought and experience of cryptogamic phylogeny, and probably no other living person has Prof. Bower's knowledge of the data or insight into the problems. The book is not like most others which can be opened or closed at any chapter and in which the data themselves have intrinsic value: it is an inductively logical construction in which no chapter or moiety stands sufficient unto itself and in which all the data have value only in comparative terms, for all move in processional and related sequence to an end. It has that symphonic quality which characterises the work of a master and, although many botanists of my generation may disagree with particular orderings of data or particular deductions, or even relegate the whole problem to the shelf, there is no one of us but is glad to acknowledge Prof. Bower as a master at whose feet we are content to sit.

WILLIAM B. BRIERLEY.

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W. B. BRIERLEY

AND

C. T. GIMINGHAM

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EXPERIMENTS WITH FOLLICULAR AND OTHER
HORMONES AND PLANT GROWTH

BY M. A. H. TINCKER, M.A., D.Sc.

*(Royal Horticultural Society, Wisley, Ripley, Surrey.)*WITH AN APPENDIX ON *BACTERIUM AUXINOPHILUM*.

BY S. E. JACOBS, Ph.D.

(Imperial College of Science and Technology, London.)

INTRODUCTION.

IN an article dealing with comparatively recent investigations concerning the action of hormones, which appeared in the medical press in 1933, Schoeller⁽¹²⁾ claimed that large differences observed between the rate of growth of experimental plants of several species, including hyacinth, lily of the valley, and arum lily, were primarily due to the action of progynon or oestrin upon these plants. Illustrations accompanying the letterpress indicated the magnitude of these differences. It was claimed that the results obtained by Schoeller and Goebel⁽¹³⁾ proved that small quantities of the follicular hormones produced marked acceleration of flowering.

In view of the possible horticultural interest these claims may arouse—for acceleration of flowering is often of considerable economic importance—it was decided to test the influence of ovarian hormones upon plant development. The tests to be described were made with compounds readily procurable in a pure condition. They were carried out at Wisley, and similar tests are being carried out at the John Innes Horticultural Institution, in which other compounds are used.

EXPERIMENTAL.

Exp. No. 1, 1932. Ketohydroxyoestrin.

Ketohydroxyoestrin can be obtained as pure crystals; it is a sparingly soluble compound, 0.1 gm. dissolves in 100 c.c. of 20 per cent. ethyl alcohol to which 2 c.c. of 10*N* caustic soda have been added. In the animal kingdom the relative rate of activity of similar compounds is a very high one; there may be approximately 10^6 mouse units in 1 gm. For this preliminary test with plants an alcoholic alkaline solution was made up to contain 0.5 mg. per c.c., or 500 mouse units per c.c.

(a) From a large number of *Bryophyllum calycinum* plants growing in a satisfactory loam in pots a homogeneous sample was carefully selected. The chosen plants were 6 in. tall, possessed four leaves, and were of the same age. Into the soft tissues of the stem just below the apical bud 0.3 c.c. of the solution was injected by hypodermic needle. Some small drops exuded and trickled down the stem to the axil of the petiole. Six control plants were injected with distilled water and there were six treated plants. The treatment caused no apparent effect upon the subsequent growth of the plants.

(b) The solution was poured on to roots temporarily exposed in the soil, in the hope that some of the ketohydroxyoestrin might be taken up by the plant. Three applications each of 0.3 c.c. were made at weekly intervals so that 0.9 c.c. (or 0.45 mg.) was presented to each plant. No difference was observed between the subsequent growth of the treated and control plants.

(c) 1 c.c. of the solution was injected into the hollow petioles of arum lilies on three occasions. There was no exudation from this plant. No significant differences were observed between the subsequent growth of the treated and control plants into which water only was injected.

(d) Plants of a cross between *Festuca pratensis* and *Lolium perenne* were available for experimental purposes; such plants have never flowered so that if hormone compounds excite a floral response the use of such experimental material afforded decidedly interesting possibilities. The solution of the ketohydroxyoestrin was poured on to temporarily exposed roots on three occasions, separated by the lapse of a week, at the rate of 0.6 c.c. to each treated plant, each plant being thus offered 0.9 mg. of ketohydroxyoestrin. No apparent effect was observed as a result of this treatment; the treated and control plants continued to bear a close resemblance to each other.

(e) Cuttings of *Berberis Neubertii* (*B. vulgaris* \times *B. aquifolium*) were placed in a weak solution of ketohydroxyoestrin containing 0.1 mg. per c.c. whilst controls were placed in distilled water. In both series slow root development took place and no difference was observed in the rate of leaf or root formation. Flower buds did not develop.

*Exp. No. 2, 1932. Theelol.*¹

Theelol is a crystalline ovarian hormone of formula $C_{18}H_{24}O_8$, of melting-point $218^{\circ}C$. It is a trihydroxy compound of greater solubility than ketohydroxyoestrin and theelin (8). It is obtained from the urine of

¹ Parke, Davis and Co.

pregnant women(6), and is standardised by biological tests made by hypodermic injection, in terms of rat units after the modified methods of Allen and Doisy(2).

(a) With a scalpel a longitudinal cut was made in the petioles of six plants of *Bryophyllum calycinum*. To the cut theelol was applied in water. Each subsequent day a further drop of water was added. Controls were made by cutting and applying drops of water only. No effects were observed other than the "healing" of the cut by the development of callus tissue.

(b) A further experiment was tried in which the theelol was mixed with pure olive oil. A small transverse cut was made in the stem just above a node near the axil of the leaf. The theelol was applied to the cut surface in the oil. In the control series olive oil only was applied. Lenticellular tissue developed on the stems below and above the node where the oil had hindered gaseous interchange. No apparent differences developed between the ten treated and ten control plants.

(c) Theelol was added to complete water cultures made up according to the Rothamsted formula. Selected small tulip bulbs, chosen by weight and also by size, were placed over the water culture solution contained in darkened bottles. To one half of the series theelol was added at the rate of 50 Doisy units to each bottle. The solutions and hormone were entirely renewed each week. There were twelve treated plants, and twelve controls to which no theelol was given. At first there was a small difference in the rate of leaf development as measured by expansion and growth in length; the treated plants grew a little more rapidly, but 3 weeks later the two series were equal in this respect. At a later date the control series of plants were a little larger according to the measurements which were subject to statistical analysis. The whole evidence showed no material alteration of the growth rate of the first leaves developing from the small bulbs. No apparent difference was seen in the roots in number or size. It was decided to repeat the experiment using hyacinths and at the same time to increase the amount of theelol presented to the roots.

Exp. No. 3, 1934. Ketohydroxyoestrin.

(a) Ketohydroxyoestrin was dissolved in alkaline alcoholic solution as before, but this time the solution was added to water cultures. In the treated series each bottle of water culture received 0.66 mg. of the ketohydroxyoestrin; in the control an equal amount of alkaline alcoholic solution without the ketohydroxyoestrin was added. Small selected

pieces of the rhizome of lily of the valley were supported by cotton-wool over the culture solution in darkened bottles. The rate of growth made by the plants of the two series did not reveal any significant differences attributable to the ketohydroxyoestrin.

(b) A similarly conducted test made with lettuce (var. Sutton's "Nonsuch") in which the plants were offered 0.33 mg. of ketohydroxyoestrin weekly gave similar negative results. There were twelve treated and twelve control plants.

In both these experiments in which water cultures were used, despite the usual precautions of sterilisation of vessels and water and despite the frequent changes of the cotton-wool plugs, it was observed that cotton-wool moistened by contact with solutions containing ketohydroxyoestrin permitted development of bacterial slimes very rapidly, frequently in 24 or 36 hours. This was more rapid than in the case of similar cotton-wool moistened by contact with the control water culture solution. It appeared as if the presence of the ketohydroxyoestrin favoured or even stimulated bacterial growth. As the infections were made by chance the collection of more accurate data was precluded.

Exp. No. 4. Ketohydroxyoestrin and theelol.

Hyacinthus, variety "Mary".

From a commercial sample of hyacinth bulbs a careful selection was made by weight and size. In October the root development was successfully encouraged by placing the bulbs over water in darkness for a period of 3 weeks. The plants were then placed in three series.

(a) Ketohydroxyoestrin: 0.25 mg. offered to each plant weekly for 6 weeks. Reaction of solution 8.5 pH.

(b) Theelol: 100 Doisy units offered to each plant weekly for 6 weeks, then 200 units for two further weeks. Reaction of solution 7.2 pH.

(c) Control: given the alkaline alcoholic solution of (a). Reaction 8.5 pH.

The solutions were renewed weekly.

The growth of the plants was somewhat irregular in that some showed elongation and development of their leaves before others of the same series. For this reason each plant was observed as a separate individual, and it was found that differences observed at the outset were maintained during the experimental period.

Measurements of the leaves and flowering shoots were made at frequent, almost daily, intervals and were subjected to statistical analysis. No evidence became available to show that the growth of the

hyacinths was accelerated by the added compound; on the contrary, the addition of the theelol retarded the rate of foliar development. Inspection proved the roots of plants in this series (*b*) to be particularly short, and further examination revealed that they were enclosed in a thick bacterial envelope of a slimy nature. No such infection was observed in the other series (*a*) or (*c*) where the reaction of the solution was more alkaline. The occurrence of this bacterial development at the lower, more acid, reaction suggests that it was a direct result of the theelol. Similar precautions were taken in all three series with regard to sterilisation of the water used.

Exp. No. 5, 1935. Auxin.

A preliminary test was made of a residue extracted from yeast and believed to be rich in auxin. The "*J*" fraction, insoluble in petroleum ether, was prepared, according to the method of Knight and Fildes (9), by the British Drug Houses. The extract was taken up in a weak alkaline solution, *N*/50 NaOH, and offered to the roots of experimental plants in that form.

Hyacinths, variety "Miniature Red", were used; the bulbs were carefully selected by size and weight so that a uniform sample was obtained. Before the experimental period of growth commenced their root development was successfully encouraged by placing the bulbs over distilled water in culture solution bottles in the dark for a period of 3 weeks. The plants were then arranged in three series:

(*a*) Auxin added in *N*/50 NaOH, 2 mg. to each plant weekly for 5 weeks—10 mg. total.

(*b*) NaOH added weekly in amount equal to that of (*a*) to distilled water.

(*c*) Distilled water only.

Generally the differences observed between the plants of the series were very small; the degree of homogeneity of the series was highly satisfactory. No significant differences in the rate of development were observed between the series. It appeared therefore that the auxin or its sodium derivative was not taken up from the solution, or was without effect upon the rate of development. No effect was apparent when the root systems were examined.

Further work with extracts containing auxin is highly desirable in view of the influence this compound has been proved to have upon the rate of cell elongation. With flowering plants a technique must be developed which will ensure that the auxin reaches the growing tissues.

The present experiment is reported to prevent other investigators wasting valuable extracts in weak alkaline solutions.

Exp. No. 6. Auxin—bacterial development.

A test was devised to observe the influence on the rooting of cuttings of an auxin extract from yeast—the petroleum ether insoluble “J” fraction. After the method used by Snow and Le Fanu (14) gelatine was selected as a medium for holding the extract against the stem and at the same time keeping the cut stem moist. Cuttings were taken from plants of *Calceolaria integrifolia* which grew in a cool house and provided sufficient suitable material for experimental purposes.

A gelatine medium of 12.5 per cent. concentration was prepared and divided into two halves. To one half, when the medium was nearly cold, 50 mg. of the extract previously taken up in weak $N/50$ NaOH was added, excess alkali being quickly neutralised by $N/50$ H_2SO_4 using an external indicator. To the control half of the medium an equal amount of NaOH as to the treated half was added and again neutralisation was carried out by dilute H_2SO_4 . The reaction of both samples was pH 6.8. To each, thymol was added at the rate of 2 parts per 10^6 . The concentration of the auxin extract was 2.5 mg. in 25 c.c. of the gelatine medium or 0.1 mg. per c.c., or 1 in 10^4 . Into the cooling gelatine, contained in specimen tubes holding 25 c.c., one cutting was inserted so that 2–3 mm. of the stem dipped into the medium. The tubes were closed with plugs of cotton-wool. As it was expected that the thymol would prevent the growth of all fungi and bacteria the stems of the cuttings were not washed with dilute mercuric bichloride before insertion into the gelatine. The leaves were previously washed by spraying them with sterilised distilled water. The tubes were placed in a germination tank at $18^\circ C.$, so that the leaves were in light and the base of the stem and gelatine in darkness.

In 24 hours, and again at 36 hours, a most rapidly developing contamination was observed. In all the tubes containing the extract liquefaction of the gelatine had proceeded apace with some resultant opacity of the supernatant liquid. In all tubes without the extract the rate of liquefaction was very much slower, in more than half it was barely discernible, and only further observations confirmed it. The liquefaction and resultant opacity were due to bacterial growth.

I am indebted to Mr D. E. Green for the examinations of the material. He found almost a pure culture of a bacillus and on making plates was

quickly able to effect isolation. This bacillus has been given the name *Bacterium auxinophilum* by Dr Jacobs.

There was no question whatsoever that the extract greatly accelerated the rate of bacterial development, and this despite the thymol. The cuttings were removed from the experiment at an early date and so yielded no data.

Table I.

Summary of experiments to test hormones.

No.	Plant	Substance	Method	Amount presented	Result
1 (a)	<i>Bryophyllum calycinum</i>	Ketohydroxyoestrin	Injection in water (alkaline)	0.16 mg.	-
(b)	"	"	To soil, in water (alkaline)	0.45 mg. (3 × 15 mg.)	-
(c)	<i>Richardia africana</i>	"	Injection in water	1.5 mg. (3 × 5 mg.)	-
(d)	<i>Festuca</i> × <i>Lolium</i>	"	To soil, in water	0.9 mg. (3 × 3 mg.)	-
(e)	<i>Berberis Neubertii</i>	"	In solution in water	1.0 mg. in 10 c.c.	-
2 (a)	<i>Bryophyllum calycinum</i>	Theelol	To cut petiole, in water	50 Doisy units	-
(b)	"	"	To cut petiole, in olive oil	50 Doisy units	-
(c)	<i>Tulipa</i> var. "Clara Butt"	"	In culture solution	50 Doisy units per week	-
3 (a)	<i>Convallaria majalis</i>	Ketohydroxyoestrin	In culture solution	0.66 mg.	-
(b)	<i>Lactuca sativa</i> var. "Nonsuch"	"	"	0.33 mg. per week	-
4 (a)	<i>Hyacinthus orientalis</i> variety	"	In solution (alkaline)	0.25 mg. per week	-
(b)	"	Theelol	In solution water	100 Doisy units weekly for 6 weeks. 200 for 2 weeks	-
5	<i>Hyacinthus</i> var. "Minia-ture Red"	Petrol ether insoluble "J" extract believed to contain "Auxin"	In alkaline solution	0.25 mg. per week for 5 weeks	-
6	<i>Bacterium auxinophilum</i>	"	In gelatine	2.5 mg. in 25 c.c. 12.5 % gelatine +	+

DISCUSSION.

From the experiments described, which are summarised in Table I, no evidence of the stimulation of the growth rate of flowering plants has been collected. It must be clearly stated and understood that it has not been proved that the substances presented to the plant, even by in-

jection, have been taken up by the tissues. It is possible that the amount presented never attained the threshold of the reaction level, despite the fairly high rates of application. No useful purpose would be served by a detailed consideration of these negative results; suffice it to say that the ovarian hormones do not appear to influence the rate of plant growth very readily.

When Schoeller's claim that flowering is accelerated by hormones is carefully examined it is seen that in several of his experimental plants, such as the hyacinth, the flower primordia are laid down before the period of experimentation. Schoeller's work was therefore concerned with an increased rate of stem elongation, no doubt a resultant of cell division and elongation. Now Went⁽¹⁵⁾, ⁽¹⁸⁾ has shown that auxin influences the rate of cell elongation⁽¹⁷⁾. It therefore appears that Schoeller really claims for other hormones an effect similar to that produced by auxin⁽¹⁰⁾. The present experiments do not substantiate this claim.

Knight and Fildes⁽⁹⁾ working with *Bacillus sporogenes* found that an accessory growth factor was necessary for the rapid development of the bacteria. They identified their "vitamine" with auxin, already known to accelerate certain phases of growth in the life-history of the cell of plants. In the growth of the bacteria it may be that auxin accelerates more than one phase, or that the phases of cell division and cell elongation are not so clearly defined. The experimental work here described afforded, quite possibly, some confirmation of their results with auxin. General observation showed that ketohydroxyoestrin and theelol may also favour bacterial growth.

SUMMARY.

In the experiments described the writer failed to obtain accelerated growth or acceleration of flowering by presenting small quantities of ketohydroxyoestrin and theelol to flowering plants in different ways—in solution given to the roots, or by injection, or when applied to cut surfaces.

From one experiment, designed for another purpose, possible confirmation of the results of Knight and Fildes was obtained in that auxin accelerated bacterial growth.

ACKNOWLEDGMENTS.

The writer expresses his thanks to D. E. Green, M.Sc., Mycologist, for assistance in isolating bacteria, and to Dr S. E. Jacobs for kindly describing the bacteria. He desires to thank Messrs British Drug Houses for their gift of the yeast extract believed to contain auxin.

APPENDIX.

BY S. E. JACOBS, PH.D.

(From the Bacteriological Laboratory, Imperial College of Science
and Technology, London.)

The organism reported by Tincker as having been stimulated by auxin was investigated, and it was found that it was apparently an undescribed species. The importance of the claim made for the organism is held to justify the introduction of yet another bacterial species into the literature, and the name *Bacterium auxinophilum* has been selected for it.

Bacterium auxinophilum.

Morphological characters. The organism is a short rod with rounded ends, occurring both singly and in pairs. Size $0.7-1.4 \times 0.5-0.7 \mu$. It is actively motile, having a single polar flagellum.

Staining. The organism stains well with carbol fuchsin and gentian violet. It is Gram negative.

Cultural characters. On a slope culture of nutrient agar at 25°C . (pH 7.0) the growth in 2 hours was luxuriant. It was moist, transparent, homogeneous, brownish by transmitted light and greyish white by reflected light. The growth was raised, the surface shining and smooth and the margin entire.

Well isolated surface colonies on nutrient agar were circular and attained in 4 days at 25°C . a diameter of 5-6 mm. Subsurface colonies were lenticular, brownish and denser than the surface growth. Cultures on nutrient agar became brownish yellow in 10 days, and discharged a brownish yellow pigment into the medium.

Nutrient gelatine stab cultures at 20°C . showed rapid liquefaction, the shape of the liquefied portion being at first saucer-shaped, later becoming saccate. Growth occurred along the whole length of the stab.

Nutrient broth was uniformly and heavily clouded, with a thick sediment, and only a slight pellicle.

The organism grew well in 24 hours at all temperatures from 20° to 37°C ., with the best growth at 25° to 30°C .

Physiological characters. Cultures for these tests were maintained at 25°C .

Glucose was rapidly fermented in 48 hours with the production of much acid but no gas. Growth in the glucose broth was scanty and the acid produced apparently restrained it. There was no bleaching of the litmus.

Lactose was fermented in 48 hours with the production of slight acid, but no gas. The growth here was very good, with a heavily flocculent sediment and a thin patchy pellicle. The litmus was wholly bleached in 10 days.

Sucrose was fermented in 48 hours with the production of acid but no gas. Growth was good, but there was no pellicle and only slight bleaching at the bottom of the culture tube.

Nitrate was rapidly reduced to nitrite in 48 hours. No gas was evolved.

In litmus milk acid was formed sufficient to form a curd which subsequently contracted, allowing the whey to separate. There was no digestion of the casein.

Indol was formed abundantly in 48 hours.

The formation of acetyl-methyl-carbinol could not be demonstrated, but the failure may have been due solely to the small amount of growth in the presence of glucose.

Growth on potato mush agar was very scanty. This medium was made from old potatoes. Growth in potato extract agar made from new potatoes was moderate but not nearly so abundant as on nutrient agar. There was no growth on raw potato or raw turnip slices.

Reactions of the organism towards auxin. Tincker's experiments were repeated and it was found that auxin from the same source as Tincker's material and in the concentration used by him, namely 1 mg. in 10 c.c., did undoubtedly stimulate the growth of the organism in plain gelatine. However, in concentrations below this, *i.e.* from 0.1 mg. down to 0.00001 mg. per 10 c.c., there was no such stimulation. Knight and Fildes(9) found that auxin stimulated the growth of *B. sporogenes* at a concentration of 0.0002 mg. per 10 c.c., and in view of the disparity between the sizes of the minimum stimulating doses in the two cases, it should not be too hastily assumed that auxin is definitely acting as a vitamin or accessory growth substance for *Bacterium auxinophilum*. Slow growth did occur in the medium alone without auxin, so that the stimulation effect may have been due solely to the provision of additional food material with the auxin concentrate. It remains to be determined whether *Bact. auxinophilum* is capable of growth in a purely synthetic medium where the nutrients, as distinct from accessory growth factors, are known to be adequate

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WILT, STEM ROT, AND DIEBACK OF THE PERPETUAL FLOWERING CARNATION

BY G. M. WICKENS, B.Sc. (AGRIC.), PH.D.

(From the Department of Plant Pathology, Imperial College of
Science and Technology, London.)

(With Plates XXVI and XXVII and 6 Text-figures.)

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I. INTRODUCTION.

IN October 1930, the attention of the writer was drawn to a case of carnation disease in a nursery at Hanworth, Middlesex. Considerable areas of beds under glass had been destroyed, and similar losses had occurred in preceding years. Enquiries elsewhere showed that the disease, known to the growers generally under the name of stem rot, was widely

spread and that in some places very considerable damage was being caused. As the disease appeared to have been very inadequately studied and as any control measures hitherto suggested had proved to be ineffective, the subject appeared to be ripe for further investigation, both from the point of view of its etiology and of its possible control.

II. HISTORICAL.

From about the end of last century, serious losses to carnation growers, caused by a disease or diseases similar to those now under consideration, have been recorded from a number of countries. To deal first with reports from the United States of America, Sturgis (21) in 1897 described a disease of carnations causing considerable loss of some varieties and generally known as stem rot or dieback. The symptoms consisted of a yellowing of the lower leaves, which later became dry and dead, and of a gradual wilting which progressed until death ensued. The outer tissues of the stem below soil level became discoloured and disintegrated, and the wood was found to be permeated by the mycelium of a fungus. Isolations from diseased material gave a species of *Fusarium*, which produced 3-5-septate spores, pointed at both ends, slightly curved, measuring $25-38 \times 3.5-4\mu$, and coloured pale salmon pink in the mass. This species was not fully identified.

Experiments in which plants were grown in soil inoculated with the fungus after sterilisation gave inconclusive results, since some of the controls in sterilised soil also contracted disease. This outbreak of disease amongst the controls was attributed to the presence of the fungus in the tissues of the original cuttings, which came from a locality where disease was prevalent.

In the following year Stewart (20) distinguished two distinct diseases, one, which he ascribed to a *Rhizoctonia* species, involving a rotting of the stems at or just below soil level and causing the plant to wilt suddenly, and the other, caused by a *Fusarium* species, characterised by a gradual wilt with yellowing or drying of the leaves, discoloration of the wood and destruction of the cortex.

Wight (24), in 1912, stated that the disease was most serious at three critical periods in the life of the plant when sharp changes in moisture or temperature conditions occurred, viz. (a) at "benching", i.e. transference from the outdoor beds to the greenhouse; (b) at the turning on of steam heat in the autumn; and (c) at the oncoming of warm sunny days in spring. Warm cloudy weather, excessive soil moisture, and too deep planting were stated to be favourable to the incidence of disease.

A species of *Fusarium*, cultural and morphological characters of which are given, was isolated from affected plants. Inoculation experiments, which were carried out on a small scale, gave inconclusive results.

Elsewhere the problem has been studied in Africa, France and England.

In 1915, under the name of wilt or crown rot disease of carnations, Van der Bijl⁽²²⁾ described a disease which existed at that time throughout the Union of South Africa, and more particularly in Natal, where carnations were being commercially grown in the open. This was characterised by a soft rot of the cortex at the base of the stem, and by a brown discoloration of the wood. The lower leaves died, their colour changing to a sickly white, and were more upright than those of healthy plants. The upper leaves then shrivelled, and the whole plant rapidly died. From pieces of diseased tissue an unnamed species of *Fusarium*, cultural details of which are given, was isolated, and its pathogenicity proved by wound and soil inoculations. The rapid appearance of disease symptoms (which were observable four days after placing cultures of the fungus round the stems below soil level) in one experiment was ascribed to the heavy rainfall which occurred at and following the time of inoculation. As control measures were recommended the growing of one's own cuttings from healthy plants only, the destruction of diseased plants, and crop rotation.

In 1920 Small⁽¹⁸⁾ recorded from Uganda a wilt of carnations and other plants, the symptoms of which were similar to those described by Van der Bijl, with the additional feature that the disintegration of tissues below the soil surface was regularly marked by a longitudinally arranged blackened sunken soft area. A species of *Fusarium* was isolated from diseased plants which on inoculation reproduced these symptoms. It was found to be culturally similar to that isolated by Van der Bijl, and was in 1922⁽¹⁹⁾ provisionally identified by the same author as *Fusarium udum* Butler.

A similar disease was recorded by Delacroix⁽¹⁰⁾ in 1900, as causing heavy losses to growers in France. Attacked plants were arrested in growth and showed a slight wilting of their leaves. This was followed by the desiccation of some of the branches, and finally the whole plant became yellow and died. Fungal attack was stated to be localised at the base of the stem, the tissues, especially the wood, being invaded by mycelium. While many organisms were found in decomposed, brown and rotted areas of the stem, only one was persistently isolated from the green tissues. This fungus, a description of which was given, was named *Fusarium Dianthi* Prill. et Delacr. As control measures were suggested

the usual sanitary precautions and the sterilisation of the soil by formalin. Mangin (14), in a paper immediately following, considered this fungus to be identical with that hitherto known as *Fusarium roseum* Link.

In England, two papers on this subject have appeared. Dowson (11), in 1929, described a disease the characteristic symptom of which was the slow withering with loss of colour of the shoots one after another. Affected plants if left in the beds developed, under moist conditions, a soft rot of the base of the stem, but this symptom was considered to be inconstant. As distinct from the above a slow dying back of shoots from the topmost node after being "stopped" was frequently met with, but was stated to be of little commercial importance.

Microscopic examination of the collars of affected plants revealed the presence of fungal hyphae in most of the tissues from epidermis to pith, its most extensive longitudinal development being in the xylem, many of the vessels and tracheids of which were filled with a brown gum-like substance. The latter also extended into side branches, although no hyphae could be demonstrated there.

From diseased tissues Dowson isolated a number of *Fusarium* species. Of these *F. culmorum* (W. G. Smith) Sacc. was found to be able to produce both the dieback and stem-rot phases of disease when inoculated into wounds, but under normal conditions in a cool greenhouse no positive infections were obtained. Two other species, *F. herbarum* (Corda) Fries and *F. avenaceum* (Fries) Sacc., were less active and at most were found to produce a certain amount of the dieback type of disease.

Observations indicated that the disease was more prevalent under relatively moist soil conditions and during the summer months. As control measures steam sterilisation of the beds, the raising of one's own cuttings "struck" in sterilised sand, the keeping of the beds on the dry side rather than the reverse, and the maintenance of as low a temperature as possible during the summer months were recommended.

In the same year White (23) described similar diseases which he called "Wilt" and "Dieback". Of a number of *Fusaria* isolated, the following caused a death of shoots when inoculated into the bases of main limbs:

Fusarium culmorum (W. G. Smith) Sacc.

F. anthophilum (A. Br.) Wr.

F. acuminatum Ell. et Ev. emend. Wr.

F. herbarum (Corda) Fries.

F. sporotrichoides (Sherb.) was also found to be slightly pathogenic. Of these species *F. culmorum* occurred much more commonly than the

others. More rapid and complete infection by this fungus, when inoculated into the bases of main limbs, was obtained in the relatively high temperature of a cucumber house than in a cool chamber, while under similar conditions its introduction into the harder stems produced no wilt within 5 months. Stem lesions at the end of this period were, however, of greater extent in those plants kept in the cucumber house. Under conditions of good drainage, soil inoculation by *F. culmorum* produced negative results, while of fourteen plants kept in infected and water-logged soil for 14 months, six succumbed within this period.

The dieback form of disease was induced by the placing of spore suspension of *F. culmorum* on the "snags" left in the "stopping" of shoots under very moist conditions and by the pricking of spores into the cut ends under ordinary conditions. No infection was obtained by the former method in the atmosphere of a cool greenhouse.

Observations on the incidence of disease agreed with those of Dowson in that it appeared to be more prevalent under conditions of excessive soil moisture and during the summer months. White also observed the tendency for the persistence of areas of disease from one crop to the next, and the fact that beds destined to show extensive disease areas in the second and third years often showed little or none in the first year after planting.

As control measures White recommended the usual sanitary precautions, steam sterilisation of the soil and the development of resistant strains.

Similar diseases of carnations, often recorded as causing serious losses and ascribed to species of *Fusarium*, have also been observed in Belgium (15), Czecho-Slovakia (27), Denmark (2, 13), Germany (1), Greece (4), Italy (6, 16) and New Zealand (9).

III. A GENERAL SURVEY OF THE PROBLEM.

A survey of the occurrence of disease known to growers generally as "stem rot" and "dieback" under commercial conditions in England would scarcely be intelligible without an account of the normal cultural practices adopted. This will be given in the first instance for the case in which disease is absent or at any rate of so little importance that the grower is able to adhere to the practices which have hitherto yielded the best financial results. The modifications of method which have either been forced upon the grower as a result of disease or with which he is experimenting with a view to disease control will be described later.

Propagation for commercial flower production is by cuttings, the cooler months of the year, especially December, January, and February, being most generally favoured for the work. Young vigorously growing side shoots, usually about 6 in. in length, are pulled off from the parent stems and may be used as such ("heel" cuttings). Alternatively and more usually the "piping" cutting is employed, in which the lowermost internodes (which are somewhat woody and bear small leaves) are rejected and the final cut made just below a node, the leaves of which are then pulled off. The cuttings are now inserted in a layer of a medium grade of clean sharp sand of 2-4 in. depth, overlying usually a layer of clinkers, in beds, boxes, pans or pots, and subjected to a bottom heat of about 55° F. and an overhead temperature of 45-50° F.

Within 3 or 4 weeks the cuttings will have rooted and are then ready for potting on into small pots. Sometimes they are placed first into "thumbs", from which, when well established, they are transferred to "60's", but more usually they are potted at once into the larger size. The young plants are grown cool, with a night temperature of about 50° F., and are given as much ventilation and direct light as possible. When well established and growing vigorously they are "stopped" by the removal of the top growth just above a pair of matured leaves, usually at about the sixth node from the base.

From the pots the young plants are transferred, with the ball of soil intact, to the beds, usually in March, April or May, being set out as a rule 8 or 9 in. apart either way. The beds are generally from 3 to 4 ft. in width, and with wooden or concrete sides about 6 in. high, so that the surface of the soil is approximately 5 in. above the level of the paths.

Until recently such beds were almost universally used, but of late years there has been an increasing tendency among growers to separate the 5 or 6 in. of top soil from the soil beneath, with a view both to the improvement of drainage and to the control of disease. Among the methods employed may be mentioned the interpolation of a floor of drain tiles or bricks, or of a layer of clinkers, beneath the top soil. The tile or brick floor may be laid directly on the soil of the greenhouse, or may be separated from it by a layer of cement or by cross rows of bricks. It is of interest to note that similar raised benches were generally employed before the war.

As the "breaks", or side shoots arising from the main stem, attain a length of 6-9 in., they are "stopped" at about the sixth node in the same way as previously described, this practice being continued until about the first week in July of the first year in order to check the

tendency to form flower buds and to develop a bushy plant. From this time onwards the plants are allowed to flower, the shoots of various stages of development resulting from the successive "stoppings" producing a continuous succession of flowers from September of the first year of planting onwards. The only remaining routine operations on the plants themselves consist of the disbudding of the flowering shoots, the cutting of the flowers, and, during the winter months, the taking of cuttings.

During the cooler months flower production proceeds steadily but comparatively slowly, the heaviest crops being taken during the spring and summer. Although productivity may decrease somewhat during the third year of growth, the plants are usually allowed to remain in the beds for 3 years. In the third spring after planting, the old plants, with the wiring and stringing used to support them, are removed, and the houses are prepared for a fresh planting.

In the preparation of the new beds there is considerable diversity in the practices of various growers. The old soil may be retained, with or without the addition of dung and artificial fertiliser. Alternatively the top 5 or 6 in. of soil is replaced by fresh soil, which may be either cultivated soil from the outdoor part of the nursery or virgin soil from old meadow or pasture land. With this is incorporated a proportion of dung (old or fresh cow or horse dung) varying from comparatively little to as much as one-third of the mixture, some artificial fertiliser, and, in the case of heavy soils, usually a proportion of burnt clay or mortar rubble. Steam sterilisation of both old soil and new soil beds is sometimes practised, and is believed by some growers to pay for itself by the resulting increase in fertility, and by the destruction of weed seeds and insect pests.

The incidence of the disease known generally to the grower as "stem rot" (a name which will for convenience be retained in the present section) has, however, in many cases necessitated modifications of the cultural methods described above.

Even in nurseries where losses in the beds are serious the writer has never seen any appreciable disease among cuttings in the sand, at least a 95 per cent. "strike" (*i.e.* 95 per cent. successfully rooted) being usually obtained. In most cases also failure to root is due to unfavourable conditions, although fungal attack, resulting in a rotting of the base of the cutting at or below sand level or a successive wilt of leaves with brown discoloration of the vascular system, is occasionally seen.

Among young plants in pots, however, losses due to "stem rot" are not uncommon, amounting occasionally to perhaps 20 per cent. among

some varieties, but such losses are usually allowed for during propagation and are, in comparison with the loss of plants in the beds, of no economic importance.

Except in the uncommon event of the planting up of beds that contained appreciable diseased areas without soil sterilisation or replacement, disease during the first year is not usually extensive, being generally restricted to a few plants scattered here and there throughout the beds and rarely spreading appreciably. On the other hand, at least one case has been reported to the writer in which almost complete destruction of the crop took place during the first year of planting. In the spring and summer of the second year, however, a rapid spread from these foci, and also from new centres which may develop, occurs. The rate of extension of the disease areas thus set up decreases somewhat during the following winter, but increases again during the hotter months of the third year. The diagrams (Text-fig. 1) constructed from weekly observations at a nursery illustrate these points.

A survey of 2-year-old plants of "Wivelsfield White" at the same nursery showed that the percentage loss in various beds ran from 7.0 to 35.2, and that altogether 3120 plants were missing from an original stand of 15,345, *i.e.* a loss of just over 20 per cent. This illustration is in no way exceptional and worse cases have been seen.

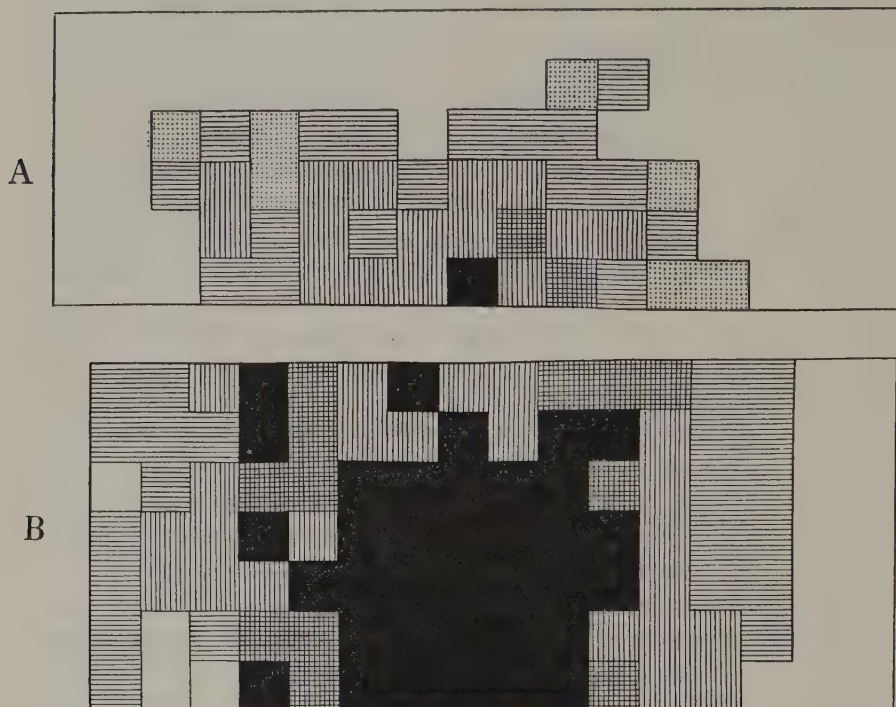
The bare areas due to "stem rot" constitute, at any rate as far as carnation production is concerned, an almost total loss to the grower, since replants almost invariably die within a month or two. In many cases the extent of disease at the end of the second year after planting is sufficiently serious to render the retaining of the bed for a third year uneconomic. Over and above the direct loss caused by the death of plants there is the loss arising from the more frequent occurrence of the unproductive period which extends from the grubbing up of the old crop to the flowering of the new one. The additional expense of renewing the beds at 2-yearly in place of 3-yearly intervals is also an important consideration.

Not all nurseries are thus seriously affected, and it is interesting to note that although, in two cases known to the writer, "stem rot" undoubtedly occurs, its spread is much less severe. One of these also reported in June 1931 that replants rarely succumbed to disease, although since that date this has not held good. The question of this comparative freedom from "stem rot" experienced by some growers will be discussed in a later section.






As stated by Dowson (11), losses due to "stem rot" in this country

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have only reached serious proportions since the war. It is of interest to note that the increase in the severity of the disease appears to have followed the abandonment of raised benches in favour of beds "on the



Text-fig. 1. Illustrating spread of disease. A, bed of variety Spectrum, planted in spring 1930; B, bed of variety Mrs A. J. Cobb, planted in spring 1929.

-  Plants diseased at commencement of observations, 2nd week in November 1930.
-  Plants that contracted "stem rot" from 2nd week in November 1930 to the 4th week in February 1931.
-  Plants that contracted "stem rot" from 4th week in February 1931 to the 4th week in May 1931.
-  Plants that contracted "stem rot" from 4th week in May 1931 to the 4th week in August 1931.
-  Plants that contracted "stem rot" from 4th week in August 1931 to the 4th week in November 1931.

Each small square represents one plant.

solid". The effectiveness of the raised bench as a means of disease control will be discussed in a later section.

It has also been suggested that the trouble has been aggravated by the greater susceptibility of the newer more productive varieties. While

it is true that some comparatively recent varieties, outstanding in vigour of growth and productivity, have proved to be extremely susceptible, further evidence is needed before it can be said that such correlation exists.

IV. THE ETIOLOGY OF WILT, STEM ROT, AND DIEBACK.

A. WILT AND STEM ROT.

(1) *The fungi isolated from diseased plants.*

After a number of preliminary trials, the following was adopted as the standard method of isolating the fungi growing within the tissues of affected plants.

The roots are removed from the main stem or collar, and the side branches trimmed off flush with the stem surface. A suitable piece of stem, usually about 5 cm. in length, from the base upwards, is washed in running water, allowed to dry, and then externally sterilised by dipping into 95 per cent. alcohol and flaming this off. The stem is then divided longitudinally by a sterile scalpel, and the two halves placed, cut surface downwards, on plates of malic acid agar of the following composition:

Malic acid	5 gm.
Agar	20 gm.
Distilled water	1 litre

During the period August 1931 to July 1932, diseased carnation plants from two nurseries were examined by the above technique. The plants varied from 6 to 18 months old, and all showed the symptoms recorded by Dowson (*loc. cit.*), without, however, a generalised rotting of stem tissues. The number of times various *Fusarium* species and combinations of the same were isolated was as follows:

<i>Fusarium</i> of section <i>Elegans</i> alone	19 times
„ „ „ + <i>F. culmorum</i>	6 „
„ „ „ + <i>Fusarium</i> of section <i>Roseum</i>	3 „
<i>F. culmorum</i> alone	16 „
„ + <i>Fusarium</i> of section <i>Roseum</i>	0 „
<i>Fusarium</i> of section <i>Roseum</i> alone	8 „

As the earlier inoculation experiments, carried out with the various species isolated in the manner indicated, give very little success, the suspicion arose that one or more of the fungi constantly isolated might be mere secondary invaders of the cortical tissues. The technique of isolation was therefore modified, viz. by the removal of all tissues external

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to the wood after sterilisation of the surface, so that the only tissues plated were xylem and pith.

With plants derived from the same nurseries and showing the same disease symptoms, a different assemblage of fungi was obtained by the modified technique. Thus a total of 120 plants examined during the year 1933 gave the following record:

<i>Verticillium cinerescens</i> alone	107 plants
„ + <i>F. culmorum</i>	5 „
„ + <i>F. herbarum</i>	1 „
„ + unidentified <i>Fusarium</i> species	6 „
<i>F. herbarum</i> alone	1 „

Thus in all but one of the 120 plants examined, *Verticillium cinerescens*, usually alone but sometimes in combination with *Fusarium* species, was isolated. This fungus was first described from the wood of wilting carnations in Germany in 1929 and provisionally named as above by Wollenweber⁽²⁵⁾. On the isolation plate it grows relatively slowly, so that in the presence of rapidly growing *Fusarium* or other contaminants it is liable to be suppressed. Hence, no doubt, the failure to observe its presence in the isolation plates made according to the earlier technique.

During 1934 isolations were made from diseased plants of various ages and varieties sent in from sixteen different nurseries. The great majority of plants from all but one of these yielded *Verticillium cinerescens*, pure or in association with species of *Fusarium* (chiefly *F. culmorum* and *F. herbarum*). One nursery proved to be exceptional in that three successive samples of plants, totalling nineteen in all, invariably gave the fungus *Fusarium Dianthi* Prill. et Del.¹ to the exclusion of the *Verticillium*. The fungus *Fusarium Dianthi* has occasionally been isolated from plants of other nurseries, occurring alone but more frequently associated with *Verticillium cinerescens*. For reasons that are not clear, it has, apart from the particular nursery mentioned, been met with less often in the later phases of the investigation than earlier on.

(2) *The pathogenicity of the isolated fungi.*

Four organisms, viz. *Fusarium culmorum*, *F. herbarum*, *F. Dianthi* and *Verticillium cinerescens*, were considered to be of sufficient importance to justify extensive inoculation experiments. The carnation plants were tested at three phases of growth:

- (i) When the cuttings were inserted in the sand.

¹ Identified by Dr H. W. Wollenweber.

(ii) When rooted cuttings are transferred from the sand to small pots (60's).

(iii) When the young plants had become established in larger pots or in beds.

Apart from special cases to be noted in the text, where special treatment such as over- or under-watering was required, all the experimental plants were subjected to the normal cultural routine of the nursery.

The experiments will now be described according to the fungi used and in the order of the list given above.

(a) *Fusarium culmorum*.

(i) *Experiments with cuttings at the time of their being placed in sand.* A typical result is shown in Table I. Contamination of the sand was brought about by adding to it macerated cultures of *F. culmorum* grown on a mixture of sand, rolled oats and water. An equal amount of the oats was added to the sand for the control batch.

Table I.
Var. Wivelsfield White.

Box	Treatment	No. of cuttings	Plants infected within		Plants which rooted
			4 weeks	6 weeks	
A	Sand contaminated	98	98	—	0
B	Basal cut surface of each cutting inoculated	98	11	78	82
C	Controls	98	0	0	98

Within 4 weeks every cutting in box A and a few of those in box B were soft and completely rotted at the base of the stem up to a point at or above sand level.

After 6 weeks the remaining cuttings of boxes B and C were removed from the sand. Box B showed all grades of infection from no visible attack to a complete soft rot of the base, so that successive stages in the destruction of the cutting could be observed. The earliest symptoms discernible were a rotting of the bases of the leaves below sand level, which became soft and watersoaked in appearance, and a slight brown discoloration round the nodes from which they arose. Later, a brown soft rot spread up and down and across the stem, from the points of exit of the leaf midribs, its development being more rapid in the longitudinal than in the transverse direction. Eventually the whole of the cross-section of the stem became soft and rotted, and the plant collapsed and died.

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Although in many cases the inoculum was found, even after 6 weeks, to be still adhering to the cut surface, no rotting from this point was apparent. In every case except those in which rotting was too far advanced from its origin to be determinable it was seen to be spreading from a node below sand level.

F. culmorum was regularly reisolated from stems showing all grades of infection and also from rotted leaf bases.

Twelve rooted cuttings from each of boxes B and C, the former having one or more rotted leaves at the base and one or more nodes slightly discoloured, were potted on into "60" pots in the normal way and left under ordinary conditions. All developed into normal healthy plants, showing no pathological symptoms within 4 months.

A similar experiment with cuttings of an unnamed seedling variety showed that 100 per cent. of infection was obtained within 2 weeks when the inoculum was inserted through a nodal wound, whereas only about 60 per cent. of infection took place within 6 weeks when the inoculum was merely placed in the axils of the lowest leaves.

The method of inoculation through nodal wounds was used frequently in the course of this investigation and was carried out as follows. A longitudinal incision was made in the stem at the lowest leaf-bearing node, through the midrib of one of the leaves arising from it. Into this incision was introduced a small piece of a culture of the fungus grown on potato dextrose agar at room temperature.

From the above results it is concluded that:

(1) Cuttings planted in sand heavily contaminated with *F. culmorum* rapidly succumb to a basal soft rot.

(2) The rotting of the tissues begins at the leaf-bearing nodes below sand level, infection of the stems apparently occurring through the bases of the leaves.

(3) Cuttings not sufficiently attacked to prevent root development can resist the advance of the parasite and develop into normal healthy plants.

(ii) *Experiments with rooted cuttings on transference from sand to small pots.* A typical set of results is shown in Table II. The method of inoculation in batch A was through a node as already described. In the case of batch C the potting soil was previously contaminated by the admixture of cultures of the fungus grown on a mixture of sand, chopped carnation shoots and water.

For the first fortnight after the potting all were given normal watering to allow the plants to become established. The thirty pots with inoculated

soil (batch C) were then divided into three groups of ten pots each, of which one group was given excessive watering, a second was watered normally, and the third given only just sufficient water to maintain the turgidity of the plants. The controls were similarly divided into three groups of four plants, each of which received the same differential treatments.

Table II.

Var. Mrs A. J. Cobb.

Batch	Treatment	No. of plants	No. of plants showing, within 3 weeks of inoculation, infection grades			No. of plants infected within 2 months of inoculation
			+	++	+++	
A	Wounded, inoculated	30	2	8	20	30
B	Wounded, not inoculated	12	0	0	0	0
C	Unwounded, inoculated	30	0	0	0	0
D	Unwounded, not inoculated	12	0	0	0	0

The infection grades were arbitrarily chosen as follows:

+ Stem showing a narrow brown streak extending upwards from the inoculated node but not reaching the node above.

++ Stem and leaves shrivelled and rotted up to the second node above the inoculation wound.

+++ Stem and leaves shrivelled and rotted up to at least the third node above the inoculation wound.

In slightly attacked plants (grade + of Table II), rotting was also seen to progress downwards in the form of a V-shaped lesion, the apex of the V being directly below the point of inoculation. The tissues of the leaf bases arising from the inoculated node were destroyed, the attack progressing round the node from the wound. With greater intensity of attack (grades ++ and +++) the internode beneath the point of inoculation was completely rotted, as were also a certain number of internodes above. In all cases complete rotting ceased abruptly at a node, but above this point two narrow longitudinal brown to purple-brown stripes, one on either side of the stem, continued through the next internode and into the midribs of the leaves immediately above.

From pieces of stems of affected plants taken from above the inoculated node *F. culmorum* was successfully reisolated.

At the close of the experiment, 2 months after the potting of the cuttings, all of the controls and all those in contaminated soil were free from pathological symptoms.

The pathogenicity of *F. culmorum* when inoculated into nodal wounds of carnations at this stage has been confirmed by tests with a number of strains of the organism and on a number of varieties (Mrs A. J. Cobb, Mrs High Reilly, Salmon Spectrum). A high degree of attack, approximating to 100 per cent. of the plants used, has been obtained within 3 weeks from inoculation with strains isolated from the following diverse sources: wilted carnation, air of a carnation house, wilted *Eryngium* plant growing in old carnation soil, soil of a bed in which 2-year-old carnation plants were growing and showing no symptoms of disease, discoloured sample of imported butter, and a piece of bone. Less severe attack was obtained with strains isolated from oats (Denmark) and wheat (England). By way of contrast non-pathogenic strains were obtained from the air of a carnation house and also from wilted carnation plants.

In all cases the symptoms produced were identical with those previously described, except that the discoloured streaks of the internode above the rotted length of stem were not invariably present, and where they did occur in the white-flowered variety Mrs Hugh Reilly and the pink Salmon Spectrum they were of a pale to medium brown in contrast with the dark brown to purple-brown stripes in the dark red flowered Mrs A. J. Cobb.

Throughout this work it became apparent that the pathogenicity of strains of *Fusarium culmorum* was correlated with their tendency to produce aerial mycelium, so that mycelial types were pathogenic whereas heavily sporing or pionnotal types were not. In a particular test of this point, two strains A and B, mycelial and pionnotal respectively, were used. Both cultures were on potato dextrose agar (P.D.A.) slopes. By the transfer of spores of B to rice (1 gm. rice, 3.5 c.c. water) a little aerial mycelium was developed. Successive transfers of aerial mycelium to further rice tubes eventually gave rise to a culture which, on being transferred to P.D.A., remained in the mycelial condition. Parallel with this the pionnotal condition was maintained by the continuous transfer of spores, so that finally mycelial and pionnotal cultures of B on P.D.A. were obtained. In a similar way mycelial and pionnotal cultures of A were developed by successive transfers of mycelium only and spores only respectively. When nodal wound inoculations were made in the usual manner on young rooted cuttings (var. Lady Northcliffe) ready infection was obtained by the mycelial cultures but not by the pionnotal.

The development of a pionnotal strain from a mycelial and *vice versa* is interpreted by the writer along the lines given by Brown(7) for a similar behaviour of *F. fructigenum*. Both the original strains used,

A and B, were observed to saltate, and the methods of cultivation adopted served merely to select the one type or the other.

The influence on infection of the point of inoculation was also examined, the conclusion reached being that internodal inoculation was much less effective than inoculation at a node.

The results of the foregoing studies on the pathogenicity of *F. culmorum* to rooted cuttings on transference from sand to small pots may be summarised thus:

Inoculation of the base of the stem through nodal wounds resulted, under normal conditions, in a complete basal rot and the death of the plant, usually within 1 month.

This pathogenic effect was produced by strains of the fungus from a wide variety of substrata.

Pionnotal strains of the fungus derived from virulent mycelial strains were non-pathogenic.

Wound inoculation of the lowest internode was less effective than the standard method of nodal inoculation.

Soil contamination gave negative results under conditions of excessive, normal, and subnormal soil moisture.

(iii) *Experiments with established young plants in pots and beds.* Altogether about 150 plants were inoculated, at one time or another, through nodal wounds below soil level with a number of strains of *F. culmorum*. The results were fairly similar in each experiment. The following is a typical example.

A batch of forty young potted plants of the variety Wivelsfield White was inoculated in the standard way, together with twenty-eight wounded plants as controls. The plants were then grown on under normal conditions. After 14 weeks, when the experiment was concluded, all of the plants were perfectly healthy in outward appearance, though on examination near the region of the wound, a slight amount of internal browning was observable. Pieces of stem, including the inoculated node, were taken from sample plants and from these cultures of *F. culmorum* were recovered. These were proved to be pathogenic to cuttings at the time of transference from sand to small pots, thus showing that the failure to attack established plants was not due to any deterioration of the fungal cultures used.

In other experiments, somewhat greater internal damage was shown in some plants. Even though they appeared to be growing in a perfectly healthy manner, there was a certain amount of cortical rotting round

the wound, and in some cases a rotting of the pith over as much as five internodes. This rotting ceased abruptly at a node. Upwards and downwards from the wound a narrow strip of the xylem was blackened and disintegrated. This black lesion extended in no case for more than 5 cm. above the wound, and was continued for a further short distance as a brown discoloration due to the clogging of some of the vessels by a brown gum-like substance. In the case of only five plants was the amount of cortical and pith rotting so serious that death resulted. This happened over a period of 1-6 months from the date of inoculation.

In a number of experiments established young plants in small pots were transferred, with the ball of soil intact, to larger pots of soil heavily contaminated with cultures of *F. culmorum*, which were then kept under normal conditions except that in one case the pots were divided into three series, of which one was given excessive watering, another was normally watered, and the other given only sufficient water to maintain the turgidity of the plants. In a total of four experiments comprising fifty-one plants, exclusive of controls, no pathological symptoms were observable and no rotting of stem tissues had occurred within periods ranging from 2 to 10 months after the transfer to the inoculated soil, although in every case the strain used was later shown to be pathogenic when introduced into nodal wounds of rooted cuttings on their transference from sand to small pots.

From the foregoing it is evident that while *F. culmorum* can, under normal conditions, cause a certain amount of tissue rotting when introduced into the collars of established young plants, this rotting is rarely sufficiently extensive to cause the death of the plant or the appearance of any pathological symptoms.

The transfer of established young plants to soil heavily contaminated with the fungus has in no case resulted in any stem rotting.

(b) *Fusarium herbarum*.

Strains of this fungus isolated from wilting carnations have been inoculated into the nodes of young plants at the time of transference from sand to small pots and into the collars of established young plants, in the same way as described for *F. culmorum*.

The symptoms produced were exactly the same as those recorded for the latter fungus, although none of the three strains used proved as virulent as many of those of *F. culmorum*.

The pathogenicity of a pionnotal saltant of one strain was compared with that of the mycelial parent, with the same result as found for

F. culmorum, i.e. while the mycelial parent was pathogenic the pionnotal saltant was not.

(c) *Fusarium Dianthi*.

(i) *Experiments with cuttings on being placed in the sand.* The details of a typical experiment are assembled in Table III. The modes of contaminating the sand with the fungus, of inoculating through nodal wounds, etc. were precisely as described for *F. culmorum*.

Table III.
Var. Mrs Hugh Reilly.

Series No.	Treatment	No. of cuttings	No. of cuttings attacked within, in weeks			No. of cuttings rooted
			8	9	13	
1	Unwounded, in contaminated sand	30	23	30	—	0
2	Unwounded, in clean sand	30	0	0	0	29
3	Wounded, inoculated at node, in clean sand	15	13	15	—	0
4	Wounded, not inoculated, in clean sand	15	0	0	0	15
5	Unwounded, inoculated at node, in clean sand	15	0	0	1	14
6	Unwounded, not inoculated, in clean sand	15	0	0	0	15

From these figures it is clear that planting in contaminated sand or inoculation at nodal wounds results in the destruction of all the plants. On the other hand, inoculation at an uninjured node gave little disease. The fourteen plants of series 5 which rooted were kept under observation for over 2 years, in which time only one died.

The first symptom of attack by *F. Dianthi* was a slight wilting of one or more leaves, which were also, uniformly or in patches, of a paler green than normal and showed a slight brown discoloration of the midribs. At later stages a number of leaves wilted and became somewhat wrinkled longitudinally, chlorotic and with browned midribs, while the normal green colour of the stem had given way to a brownish colour. Finally the whole of the stem became brown, soft, and completely rotted, and the cuttings collapsed and died. In the case of nodal wound inoculations the leaves in the same vertical line as the wound were the first to become affected, and a brown streak appeared along the affected side of the stem. Soon however the whole cross-section of the stem became involved, resulting in the collapse and death of the plant.

Internally, slightly affected plants showed a brown discoloration of the vascular system which, while most strongly marked at the base, was

present throughout the length of the stem. A number of the vessels and tracheids were filled with a brown, gum-like substance, and in a few of them fungal hyphae were demonstrable. At later stages vascular browning became more intense, and a watersoaked appearance spread inwards and outwards from the xylem until the whole cross-section of the stem became soft, brown, and rotted. In the case of wound inoculations a narrow strip of the xylem first became affected, from which there spread a rot of the tissues internal and external to it.

From affected cuttings *F. Dianthi* was regularly reisolated free from other organisms.

At the earliest stages of attack, cuttings in contaminated sand showed no discoloration of the exterior of the stem nor any rotting of the leaf bases, indicating that the entry of the fungus was through the cut surface of the base, in contrast with attack by *F. culmorum* which began at the nodes. This conclusion is further strengthened by the negative results obtained when inoculum was placed in leaf axils.

From the foregoing it is concluded that *F. Dianthi* can cause the death of cuttings under normal conditions, the symptoms being a wilt of leaves with a brown discoloration of the vascular system, which is rapidly followed by a complete soft rot of all of the tissues of the stem.

(ii) *Experiments with rooted cuttings on transference from sand to small pots.* Inoculations through nodal wounds led to a wilting of nearly all the plants so treated. The incubation period was rather long, viz. from 7 to 10 weeks as compared with less than 2 weeks for a virulent strain of *F. culmorum*, under the same conditions. The symptoms produced were similar to those resulting from the wound inoculation of cuttings, described above, with the exception that during the time that the plants were allowed to remain in the pots only a slight rotting of the pith contiguous with the affected xylem occurred, and no general soft rot developed. Instead, the brown discoloration of the vascular system was followed by a "shreddy" dry rot, pale brown in colour, of the wood and the tissues external to it.

(iii) *Experiments with established young plants in pots.* Nodal wound inoculations with plants of this age have almost invariably failed to produce disease. On the other hand, in one case where eight plants of a scarlet seedling variety were planted in soil artificially contaminated with the fungus, three of them showed 14 weeks later a unilateral wilting and chlorosis of leaves of the main stem and of the breaks developed from their axils. The xylem on the side of the wilting leaves and breaks was discoloured and the cortex external to it brown and rotted. The adjacent

pith was also slightly watersoaked in appearance. In each case *F. Dianthi* was reisolated free from other organisms from every internode of the main stem. The remaining five plants in contaminated soil and the four controls remained healthy until they were discarded 10 months after the inoculation.

While it appears that under certain conditions *F. Dianthi* is able to attack and kill established young plants, further investigation is required before definite conclusions can be drawn.

(d) *Verticillium cinerescens*.

(i) *Experiments with cuttings on being placed in sand.* An illustrative set of results is shown in Table IV. The usual controls, which remained healthy throughout, are omitted from the Table.

Table IV.
Var. Mrs Hugh Reilly.

Treatment	No. of cuttings	No. attacked, in weeks						No. rooted
		8	13	20	24	26	28	
In contaminated sand	30	20	21	21	26	30	—	10
Inoculation in wounded nodes, clean sand	15	11	11	11	11	12	13	4
Inoculation in unwounded nodes, clean sand	15	0	0	0	0	0	0	15

A high percentage of infection was obtained by nodal wound inoculations or by contamination of the sand. On the other hand, no attack resulted from inoculation at unwounded nodes. The incubation period is comparatively long, so that some of the cuttings which finally succumb are able to root in the meanwhile.

The symptoms produced were similar to those caused by *F. Dianthi*, in so far as the leaves wilted and became somewhat longitudinally wrinkled and chlorotic, and a cross-section of the stem showed a brown discoloration of the vascular ring. Leaf chlorosis was, however, rather less marked, and vascular discoloration, in cuttings affected as the result of being placed in contaminated sand, tended to be localised in a number of small groups of vessels and tracheids, so that the peeling off of the cortical tissues revealed a number of longitudinal brown stripes. The most striking difference, however, was that in no case did any extensive rotting of pith or cortex appear, although a few of the plants were left *in situ* for 2 weeks or more after the appearance of leaf wilting and chlorosis.

Microscopic examination of successive cross-sections of the stem showed that the proportion of xylem elements filled with a brown

gum-like substance, and the proportion containing demonstrable fungal hyphae, decreased from the base upwards. This fact, coupled with the negative results obtained when cuttings, inoculated by placing the fungus in leaf axils, were placed in clean sand, indicates that infection occurred through the cut surface.

The history of the development of disease symptoms in one of the cuttings struck in contaminated sand is interesting in that it shows how long the plant can hold out against the disease. This cutting appeared perfectly healthy until the 13th week after being placed in the sand, when a slight wilt of one of the leaves of the fifth leaf-bearing node was just observable. Four weeks later no further advance in symptoms had occurred, except that the stem was somewhat "kinked" at the node bearing the affected leaf, the upper part leaning towards the affected side. One month later the plant appeared to be recovering for the "kink" was less striking than before and the lack of turgidity of the affected leaf was hardly discernible. The symptoms of disease were at this time so slight that they were only apparent after the most careful examination. Two weeks later however they were readily observable, one leaf of each of the first, second, third and fifth leaf-bearing nodes being slightly chlorotic and wilting. The third, fourth and fifth nodes were also abnormally crowded together, and the "kink" had quite disappeared. On this date, a cross-section of the stem showed a browning of the vascular ring on the side of the plant bearing the wilting leaves, but the pith and cortex were apparently unaffected. From this plant and a number of others of the same series *Verticillium cinerescens* was reisolated, free from other organisms.

(ii) *Experiments with rooted cuttings on transference from sand to small pots.* With plants at this stage, inoculation through nodal wounds or potting in artificially contaminated soil has, with very rare exceptions (*e.g.* in one experiment, 1 out of 66), led to a wilting of the plants. A number of different types of inocula were used such as spore suspensions, pieces of stems on isolation plates, and pieces of cultures on nutrient agars and plain agar. No appreciable difference in their virulence was found.

In the various inoculations carried out, the incubation period varied from about 7 to 17 weeks. The time of year at which the experiments are carried out is probably of importance in this connection, as indicated by the figures shown in Table V.

From the values of the function t , the probability of the significance of the differences can be calculated from the table of t given by Fisher (12).

In each case the probability is greater than 100:1, indicating that the differences observed are highly significant.

Table V.

Var. Mrs A. J. Cobb.

Method of inoculation	No. of plants inoculated	Date of inoculation	Mean incubation period weeks	<i>t</i>
Into nodal wounds	6	12. i. 34	15.8	9.42
"	6	13. iii. 34	7.7	
Soil contamination	6	12. i. 34	14.7	7.49
"	6	12. iii. 34	10.3	

Since all other factors were as nearly equal as possible, it is highly probable that the shorter mean incubation period in the later experiment was due to the higher average temperature.

The symptoms produced were similar to those developed in inoculated cuttings, with the further observation that even before the appearance of any leaf wilting or chlorosis a marked arresting of growth had occurred. In plants destined to show markedly unilateral symptoms, this stunting found expression in a striking curvature of the stem towards the side that later showed wilting leaves, while frequently it appeared to be restricted to a short length of stem (usually at about the fifth node) so that a definite "kink" was seen. This "kinking" was invariably followed by the wilting of leaves arising from the nodes at which it occurred, on the side of the stem towards which the upper part was inclined. Photographs of affected plants showing stem curvature and "kinking" are given in Plate XXVI, fig. 2.

(iii) *Experiments with established young plants in pots.* Inoculations of *Verticillium cinerescens* into nodal wounds made in the collars of established young plants have, in contrast to those of the preceding fungi, yielded consistently positive results. In one of these, forty plants, approximately 6 months old in "48" pots, were inoculated in this way in June 1933. These were divided into three series, which were inoculated with different strains of the fungus. Each series was further subdivided into two lots, of which one was kept under normal conditions in the greenhouse and the other left in the open until 22. ix. 33, 13 weeks from the beginning of the experiment. On this date the survivors of the outdoor series were brought back into the greenhouse. The results of this experiment are given in Table VI.

The three strains had been isolated from wilting plants at different times, No. 1 having been kept in artificial culture for a considerable

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period while the inocula of No. 3 were taken directly from the isolation plate. No. 2 was of intermediate age. The figures suggest the occurrence of a loss of virulence in artificial culture.

Table VI.
Var. Salmon Spectrum.

Strain of fungus	No. of plants inocu- lated	Location during first 13 weeks after inoculation	No. of plants showing wilt symptoms within in weeks							Mean incuba- tion period weeks	<i>t</i>
			7	8	11	13	16	26	36		
1	5	In greenhouse	0	0	2	2	2	2	2	—	—
1	5	In the open	0	0	1	1	1	1	2	—	—
2	5	In greenhouse	1	3	3	3	4	4	4	—	—
2	5	In the open	0	0	2	2	2	2	2	—	—
3	10	In greenhouse	4	7	7	9	9	9	9	8.7	2.8
3	10	In the open	0	2	4	5	6	7	10	20.1	
Controls	10	In greenhouse	0	0	0	0	0	0	0	—	—
Controls	10	In the open	0	0	0	0	0	0	0	—	—

In estimating the influence of the environment on the incubation period, only the results obtained by the use of the strain that gave a high percentage of positive infections are considered, and further, the one plant in the indoor series that showed no symptoms within 36 weeks is neglected, it being assumed that no infection had occurred. In the last column the value of the *t* function calculated from the data obtained is given. Reference to the tables of *t* given by Fisher⁽¹²⁾ shows that, for the number of plants used in the experiment, this value represents a probability of the significance of the difference of between 50:1 and 100:1. The greater mean incubation period of the series kept in the open is therefore highly significant, and, since all other factors were as nearly as possible equal, can only be reasonably ascribed to the lower average temperature of their environment.

The symptoms developed were a progressive wilting of the leaves of the green shoots from the proximal ends upwards, followed by the wilting and desiccation of the shoots as a whole, and a brown discoloration of the vascular system of the main stem and affected branches. An interesting feature was the frequent appearance of leaf wilting in the shoots arising from the topmost nodes of the main stems before any symptoms were apparent in the lower shoots.

The plants placed in the open made much slower growth than those in the greenhouse, the nodes remaining closely crowded and bearing comparatively rigid and upright leaves. After being replaced in the greenhouse, however, all the shoots of the controls lengthened and the leaves again assumed their normal condition, so that after a month or

two they were indistinguishable from those that had been kept in the greenhouse throughout the experiment. Those inoculated plants that later showed definite leaf wilting differed, however, in so far as the shoots that were destined first to show the symptoms of disease retained the outdoor type of growth.

From every affected plant *Verticillium cinerescens* was recovered from parts of the main stem above the inoculation wound, and fungal hyphae were demonstrable in the tracheids and vessels.

(3) *Conclusions regarding the etiology of stem rot and wilt.*

The more important results obtained from the foregoing experimental work may be summarised as follows.

Fusarium culmorum has been found, on inoculation into wounds, to cause an indiscriminate rotting of stem tissues. In the case of cuttings inoculated before being placed in the sand, and rooted cuttings on being potted on, this rotting rapidly spreads throughout the stem and causes the collapse and death of the plant. In the case of established plants, however, while appreciable generalised rotting sometimes develops, it is rarely sufficiently extensive to cause any apparent ill-effects to the plant.

Contamination of the sand by this fungus has resulted in a heavy mortality, due to a basal soft rot, of unrooted cuttings. At no stage, however, has any stem rotting been observed to occur in rooted plants as a result of soil contamination.

F. herbarum has, in the few experiments performed, given results similar to those of *F. culmorum*.

Neither of these fungi has been found to reproduce the characteristic internal feature of disease as it commonly occurs at commercial nurseries, *i.e.* a brown discoloration of the vascular system of the collar and affected shoots, with no generalised tissue rotting.

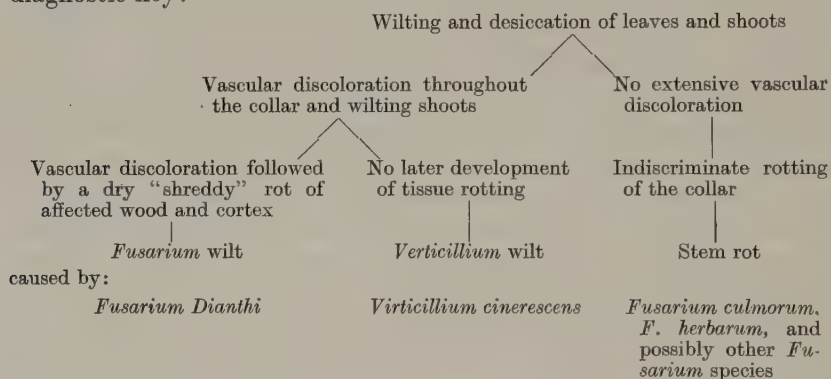
F. Dianthi, when inoculated into nodal wounds of cuttings, produces at first a wilting and chlorosis of the leaves and an extensive brown discoloration of the vascular system on the inoculated side of the plant. In the case of cuttings inoculated before being placed in the sand, these symptoms are rapidly followed by a complete soft rot of the stem with collapse and death of the plant. Similar symptoms develop when rooted cuttings are inoculated before being potted on, except that, instead of a complete basal soft rot, a dry "shreddy" rot of the wood and cortex ensue. Only rarely have these symptoms developed as a result of the inoculation of established plants.

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Cuttings placed in contaminated sand show the same symptoms as those resulting from wound inoculation at the same stage, except that the symptoms are general rather than unilateral.

Wilting and chlorosis of leaves, with a brown discoloration of the vascular system but with no later development of a rot of any of the stem tissues, have consistently resulted from the inoculation of *Verticillium cinerescens* into wounds of plants at all stages of growth, and also from the contamination of sand and potting soil by the fungus.

On the basis of general observations on commercial nurseries and the above experimental data, it is claimed that carnations under glass in this country are subject to three symptomatically and etiologically distinct diseases that have hitherto been collectively known to the growers as "Stem Rot". These diseases are named and distinguished in the following diagnostic key:



(4) *The mycology and physiology of the causal organisms.*

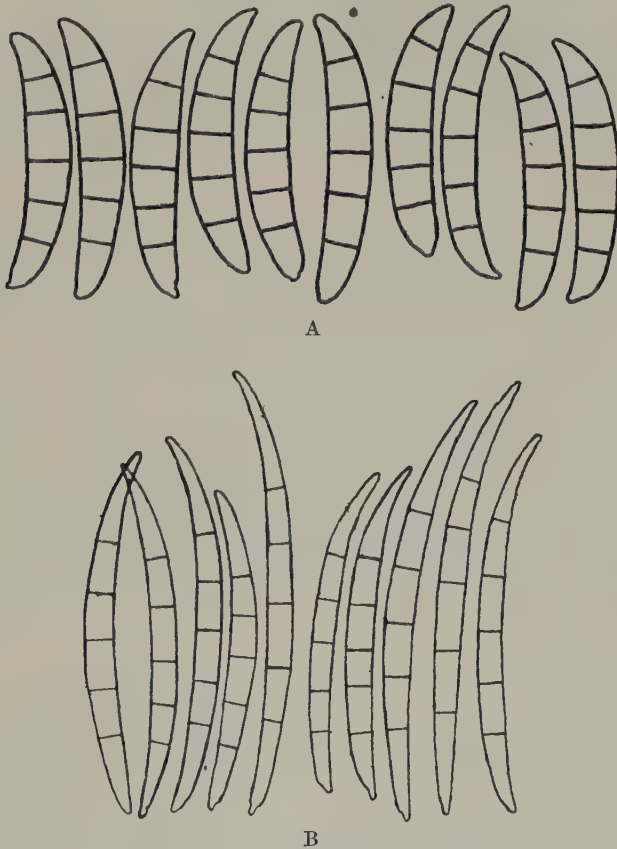
Fusarium culmorum is a fungus of common occurrence and wide distribution, and has been adequately described elsewhere (Bennett(5) and Wollenweber(26)), so that no detailed description is necessary here. Its characteristic features on the isolation plate are its lobular type of growth in the medium (shown in Plate XXVII, fig. 3), which in reflected light is Bordeaux to neutral red¹ in colour and in transmitted light carmine, and the production of numerous small sporodochia, up to 1 mm. in diameter and tawny to russet in colour, over the surface of skin and medium.

F. herbarum is similar as regards coloration of the medium, but differs in its growth on the isolation plate, which is regular rather than lobular,

¹ Ridgway's *Color Standards and Nomenclature*, 1912.

and in its sporodochia, which are larger (up to 3 mm. diam.), and ochraceous orange to apricot orange in colour.

The sporodochia of these two species bear predominantly 5-septate macroconidia, which differ greatly in size and shape as shown in Text-fig. 2.



Text-fig. 2. Illustrating conidia of (A) *F. culmorum*, (B) *F. herbarum* from sporodochia on piece of stem on isolation plate. Camera lucida drawings, $\times 1000$.

In view of the absence in the literature of any detailed description of *F. Dianthi*, the main cultural and morphological features of growth of the fungus on malic acid agar isolation plates and on potato dextrose agar (200 gm. steamed potatoes made up to 1000 c.c. + 15 gm. dextrose + 15 gm. agar) are given here in some detail.

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Aerial mycelium:

On isolation stems: white, fine cotton-woolly in texture, and somewhat flocculent to powdery on the surface, this appearance being due to numerous aggregations of freely branched hyphae consisting of rather short and swollen cells. These hyphal clusters are the beginnings of the sclerotial bodies described below.

On potato dextrose agar (P.D.A.): in cultures kept in a dim light similar to the above, but in those kept in a strong light it becomes coloured, grading from white at the tips of the hyphae through orange pink and flesh pink to deep vinaceous at the surface of the medium.

Colour produced in medium:

In isolation plates: the surface of the wood of collars plated by the modified method becomes, in parts, dusky dull violet to raisin black in colour, this colour also being present within the medium round the stem, paling outwards to deep lavender. In the case of green shoots, plated without removal of the external tissues, coloration of the medium is rather more red, ranging, from the stem piece outwards, from raisin black through deep purplish vinaceous to pale vinaceous and is sometimes seen to be concentrated in numerous narrow zones.

In P.D.A. cultures: in a thin layer immediately below the surface of the medium a bluish coloration develops, ranging from pale Russian blue to dark Delft blue or dark bluish grey green. The bulk of the medium also becomes coloured, from pale vinaceous lilac to dull Indian purple, at temperatures between 20 and 32° C.

Sclerotial bodies:

On isolation plates: regularly rounded or cauliflower-like in shape, usually 1–2 but sometimes up to 3 mm. diam., hard, brittle, and warm buff to light ochraceous buff in colour; freely formed on the surface of the stem, and also on the surface of and within the medium. Those formed by the aerial mycelium begin as the hyphal aggregations referred to above, the centres of which, with increase of size, become hard, brittle, and of a buff colour. At an early stage they therefore appear as white pustules, consisting of a hard brittle centre with a dense thin coating of mycelium. Later, with the matting down of the aerial mycelium, the mycelial coating disappears, and the mature sclerotial body is exposed.

On P.D.A.: similar to the above, but in plate cultures somewhat larger (up to 6 mm. diam.). In some plate cultures there have also developed very small sclerotial bodies (not more than 0.5 mm. diam.),

dark Chessylite blue in colour, on the surface of and just within the medium.

Chlamydospores:

On isolation plates and on P.D.A.: globose or somewhat pear-shaped, thick-walled, with a smooth or somewhat verrucose surface, $8.5 (7-11)\mu$ diam. (av. of 100), intercalary or more commonly terminal and often borne on the ends of short side branches, usually formed singly although occasionally in short chains. They occur sometimes in the aerial mycelium and invariably within the medium, in which they tend to develop in isolated patches or streaks which are macroscopically evident as light buff areas.

Sporodochia:

Up to 1 mm. diam. and capucine buff to capucine orange in colour, bearing mostly 3- and 4-septate and occasionally also 5-septate macroconidia on verticillately branched conidiophores; sometimes produced on isolation stem pieces, but not as yet on any other medium.

Pionnotes:

Light to ochraceous buff in cultures kept in a dim light, and pale to light ochraceous salmon in those in a good light; these have developed in P.D.A. cultures devoid of sclerotial bodies, consisting of conidia of septations ranging from 0-septate microconidia to (very rarely) 7-septate macroconidia, borne over layers of densely massed chlamydospores.

Microconidia:

$10 \times 3 (6-15 \times 2-4.8)\mu$ (50 from the aerial growth of a P.D.A. culture measured), produced in abundance on a number of media, being successively developed and abstricted from the tips of short side branches of the aerial mycelium and collected in small heads.

Macroconidia:

Thin walled, with somewhat indistinct septa, pedicellate, more or less attenuated or constricted at the apex 3-septate, $30.7 (25.37) \times 4.15 (3.5-5)\mu$; 5-septate, $40.5 (33-50) \times 4.3 (3.8-5)\mu$.

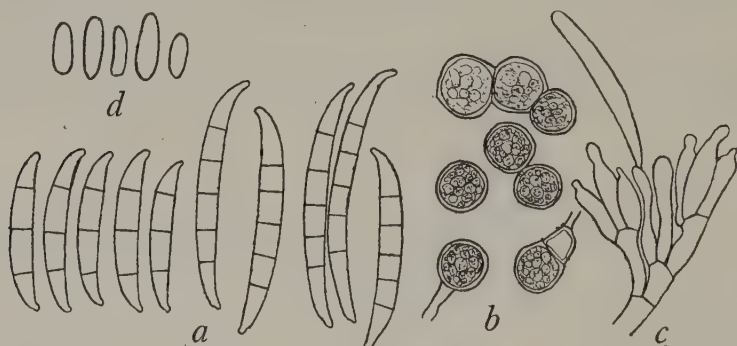
Drawings of typical micro- and macroconidia and chlamydospores of this fungus are given in Text-fig. 3.

A photograph of a typical growth of the fungus on the isolation plate is given in Plate XXVII, fig. 4.

The cultural and morphological data so far obtained, while placing the fungus without doubt in the section *Elegans*, do not justify its being

placed with certainty in any of the three subsections into which this section is now divided (Wollenweber (26)). It is, however, in fairly close agreement with the Latin diagnosis of *Fusarium Dianthi* Prill. et Del. given in Saccardo (17), and this name is therefore retained, at any rate for the time being, for the fungus under consideration. It may also be noted that, as far as they are comparable, the descriptions of the *Fusarium* species isolated by Sturgis (21) and of *F. udum* Butler (8) are in fairly good agreement with *F. Dianthi*, so that it is possible that Sturgis (21), Van der Bijl (22), and Small (18, 19) worked with the same fungus.

Verticillium cinerescens may readily be recognised in the isolation plate (Plate XXVII, figs. 3 and 5) by its tangled woolly aerial mycelium, which is pale smoke grey to light greyish olive or light drab in colour, and by its production, on the surface of the stem and both on and within



Text-fig. 3. *Fusarium Dianthi*: a, 3- and 5-macroconidia; b, chlamydospores; c, part of a sporodochium; d, microconidia. Camera lucida drawings, $\times 1000$.

the medium, of very numerous penicilli, many of which are sufficiently large to be macroscopically visible as minute olivaceous black dots.

The penicilli consist of densely massed and freely verticillately branched conidiophores, as illustrated in Text-fig. 4, terminating in whorls of sterigmata from the tips of which the conidia are abstricted.

The conidia are ovoid to ellipsoid or cylindrical in shape, olivaceous black in mass, and 4.3×2.1 ($3.6-5.6 \times 1.6-2.6$) μ in size, and collect in large masses round the penicilli on which they are borne.

Highly refractive small round bodies are frequently observed in the conidiophores, sterigmata, and in the spores, the latter usually containing one at each end.

The fungus makes good, although slow, growth on a large variety of natural or synthetic agar media. On Richards' solution agar spore production is so intense as to give the culture, almost to the edge of the

growth, a uniformly olivaceous black coloration. On other media, such as oatmeal agar, sporulation is more or less restricted to macroscopically visible (up to 0.3 mm. in diameter) olivaceous black sporodochium-like aggregates of large numbers of penicilli bearing masses of conidia.



Text-fig. 4. Conidial fructifications of *Verticillium cinerescens*; *a* and *d*, branched conidiophores; *b* and *c*, hyphae bearing sterigmata in whorls, with early stages in the formation of penicilli. Camera lucida drawings: *a*, $\times 1000$; *b*, *c* and *d*, $\times 1500$.

The following figures were obtained from the measurement of twenty conidia from each of five different media, the cultures having been grown in test tubes for 7 weeks at laboratory temperature:

Brown's synthetic medium B ...	3.1×2.5 ($2.3-4.4 \times 1.5-3.3$) μ
Oatmeal agar	4.2×1.9 ($2.8-6.3 \times 1.4-3.2$) μ
Potato tuber plugs	3.4×2.1 ($3.0-5.2 \times 1.5-2.5$) μ
Potato stems	3.6×2.0 ($2.2-5.0 \times 1.5-2.6$) μ
Elm twigs	4.9×2.0 ($3.8-8.0 \times 1.5-2.6$) μ

The linear growth rate at difference temperatures of the four species described above was tested, with the following results:

<i>Fusarium culmorum</i> ...	}	Opt. ca. 25° C.
<i>F. herbarum</i> ...				
<i>F. Dianthi</i>	„ 29° C.
<i>Verticillium cinerescens</i>	„ 20° C.

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Verticillium cinerescens failed to grow at 35° C. but was not inhibited at a temperature as low as 1.5° C. *Fusarium Dianthi*, on the other hand, grew strongly at 35° C. but not at all at 10° C. The latter fungus thus shows cardinal points for temperature which are distinctly higher than those of the former.

B. DIEBACK.

This disease is characterised by a generalised rotting of the tissues of the stem that progresses slowly downwards from a "snag" left in stopping or in the cutting of a flower, with wilting and desiccation of the leaves and of shoots arising from their axils. The plating of pieces of tissue taken from the advancing edge of the rot has invariably resulted in the isolation of *F. culmorum*.

In a number of experiments at commercial nurseries under ordinary cultural conditions, these symptoms have been reproduced by the introduction of this fungus into needle punctures of freshly made "snags", which were afterwards covered with grafting wax. In one such experiment, a number of strains of *F. culmorum* were used, each strain being tested on each of the same five varieties. Altogether 150 such inoculations were made in this case, with results very similar to those recorded on p. 644 relative to stem rot. Thus, from one-half to all of the plants inoculated were attacked by strains derived from the following sources: carnations, soil of carnation bed, wheat, daffodil bulbs; whereas attack varying from none to less than half of the plants inoculated was shown with strains derived from carnations, wheat, oats, barley and with strains obtained from two of the standard collections. The results also indicated that there were differences in resistance in the varieties tested. Thus the varieties Mrs A. J. Cobb, Lady Northcliffe and Spectrum only gave about 25 per cent. of successful inoculations, whereas with Wivelsfield White and Topsy the figures were in the neighbourhood of 70 per cent.

As with the stem-rot disease produced by *F. culmorum* experiments showed that pionnotal types of culture were much less active than mycelial ones. The former were in fact non-pathogenic.

Simultaneously with the above experiments, tests were made in the same manner with the two wilt-producing organisms, *F. Dianthi* and *Verticillium cinerescens*. Six inoculations with the *Verticillium* all produced a type of dieback whereas a like number with the *Fusarium* all failed.

The first observable sign of attack by a mycelial culture of *F. culmorum* was the more rapid shrivelling of the "snag" as compared with

the controls, together with a dark purplish brown coloration. This was followed by a wilting of the leaves of the first node below the point of stopping, and later by their desiccation and death. In some cases no further symptoms developed and in due course normal healthy breaks arose from each node. In others the dieback spread downwards as far as the second node, the stem above this point becoming rotted, shrivelled and dark brown in colour, while one or both leaves at this node wilted and died. The disease might then be arrested at the second node, healthy breaks arising at all but the topmost node; and so on. In this way the whole side shoot might be destroyed, but in no case was the disease found to enter the main stem of a well-established plant. The rate of progress was slow, the time required for the dieback to reach the second node below the point of stopping being fully 2 months.

These observations are in good agreement with those of Dowson (11) and White (23) on this subject.

The symptoms produced by *Verticillium cinerescens* differed from those described above in a number of particulars. In every case a break, at first normal and healthy in appearance, developed from the top node. With the downward progress of disease, the leaves of the main stem, from the top node successively downwards, became bright yellow but remained turgid for a considerable time before their eventual wilting and death. The leaves of each break, successively from the base upwards, showed at first a pale green mottling, followed by chlorosis which spread from the midrib outwards, and brown discoloration of the midribs. These also eventually wilted and died. The breaks from the top nodes first became thus affected, followed by those from the nodes below in succession downwards.

Seven months after the date of inoculation two or more breaks of each plant were straw coloured and dead. Internal examination showed that, from the upper completely rotted and shrivelled part of the stem, a brown discoloration of the vascular system extended downwards in some cases as far as the roots. From all parts of the stem of each plant *V. cinerescens* was reisolated free from other organisms.

From this one experiment it appears that *V. cinerescens* is able to cause a dieback type of disease. To what extent this may occur naturally is at present unknown.

V. ON THE RELATIVE ECONOMIC IMPORTANCE OF *FUSARIUM* WILT, *VERTICILLIUM* WILT, STEM ROT, AND DIEBACK.

From time to time samples of diseased plants of various ages and varieties have been sent to the writer or collected personally from sixteen different nurseries in this country. These have been examined in a routine manner, the symptoms being noted and the collars of the plants (and sometimes also in doubtful cases parts of the green shoots) then plated by the modified method (see p. 639). The results of this work are given in Table VII.

Table VII.

Nursery	No. of plants examined	No. of plants found to be affected by						
		<i>Verticillium</i> wilt (A)	<i>Fusarium</i> wilt (B)	Stem rot (C)	A + C	B + C	A + B	A + B + C
A	10	5	—	—	1	—	4	—
B	140	107	1	1	12	—	19	—
C	8	4	—	3	—	1	—	—
D	13	12	—	—	1	—	—	—
E	11	6	—	3	—	2	—	—
F	19	—	16	—	—	3	—	—
G	5	4	—	1	—	—	—	—
H	7	5	—	—	—	2	—	—
K	10	6	—	1	3	—	—	—
L	8	6	—	—	1	—	1	—
M	1	1	—	—	—	—	—	—
N	2	2	—	—	—	—	—	—
O	7	3	2	—	1	—	—	1
P	2	2	—	—	—	—	—	—
Q	3	2	1	—	—	—	—	—
R	5	2	—	2	1	—	—	—
Totals	251	167	20	11	20	8	24	1

From the above data it is evident that *Verticillium* wilt is widely distributed, and although a more extensive survey would be desirable before final conclusions were drawn, it would appear to be mainly responsible for the heavy losses to carnation growers in this country. While in general *Fusarium* wilt appears to do little damage, it is nevertheless able to assume epidemic proportions, as was found at nursery F, where it was, in the early part of 1934, causing serious losses in one or two beds of the variety Lady Northcliffe.

Plants affected with stem rot alone have only occasionally been found, although *F. culmorum* and *F. herbarum* are not infrequently associated with one or other of the wilt diseases, especially in plants showing rather advanced symptoms. This, together with the rare occurrence of the stem-rot organisms in the wood of plants in the early stages of disease, suggest that plants primarily infected by one or other of the wilt organisms often

become secondarily attacked by the organisms of stem rot. As stated by Dowson, the name of "Stem Rot" commonly employed by growers is due to this later development of a general rotting of the tissues of the collar. It is of interest to note that during the course of this work there have been found a number of plants, affected by *Verticillium* wilt and also by stem rot, in which the combined symptoms closely simulated those of *Fusarium* wilt. It is therefore not as yet possible to diagnose the latter disease with certainty from the appearance of the symptoms alone.

The writer's observations on the incidence of dieback on commercial nurseries confirm the statements made by Dowson (11) and White (23) that this disease, although of common occurrence, is of little commercial importance.

VI. THE DISTRIBUTION OF *VERTICILLIUM CINERESCENS* AND *FUSARIUM DIANTHI* WITHIN THE TISSUES OF AFFECTED PLANTS.

The distribution of the fungus within the tissues of a number of plants infected by one or other of the two wilt organisms, in relation to the distribution of wilting leaves and vascular alteration, has been examined by plating out, in the usual way, a number of internodes from every shoot. Isolations from woody lengths of stem were made by the modified method described on p. 639, while internodes from the green shoots, removed by breaking at the nodes, were plated without removal of the external tissues.

The results of such an examination of a plant showing wilting of one shoot arising from the top node of the main stem as a result of wound inoculation at the base by *Verticillium cinerescens*, but no symptoms of disease in any of the other shoots, are shown in diagrammatic form in Text-fig. 5.

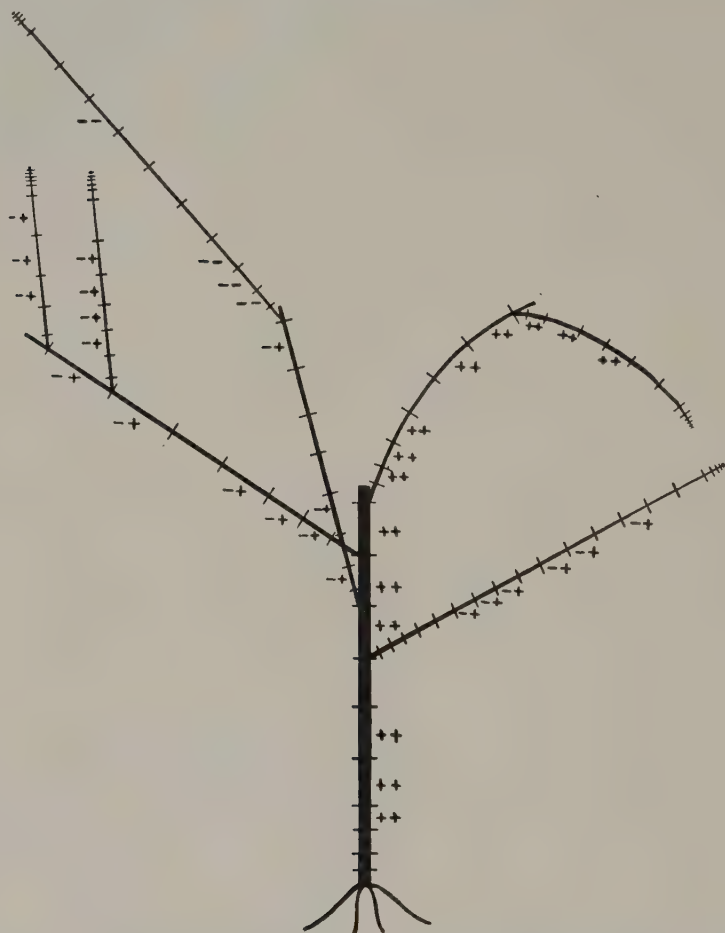
In this diagram, healthy shoots are represented by straight lines, and wilting shoots by curves.

In all diagrams in this section, the distribution of vascular alteration visible to the naked eye and of the fungus within the tissues is given by signs alongside the internodes examined, the first sign representing the presence (+) or absence (-) of vascular alteration and the second showing whether the causal organism was isolated (+) or not (-).

In Text-fig. 6 are shown the results of a similar examination of a 1-year-old plant of var. Mrs A. J. Cobb, naturally infected by *Verticillium cinerescens*, showing, in April 1934, very early symptoms. None of the

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shoots was wilting as a whole, but on some of them, apart from the natural wilt and death of the leaves of the woody main stem and lower parts of the side branches, one or more of the leaves of the green parts

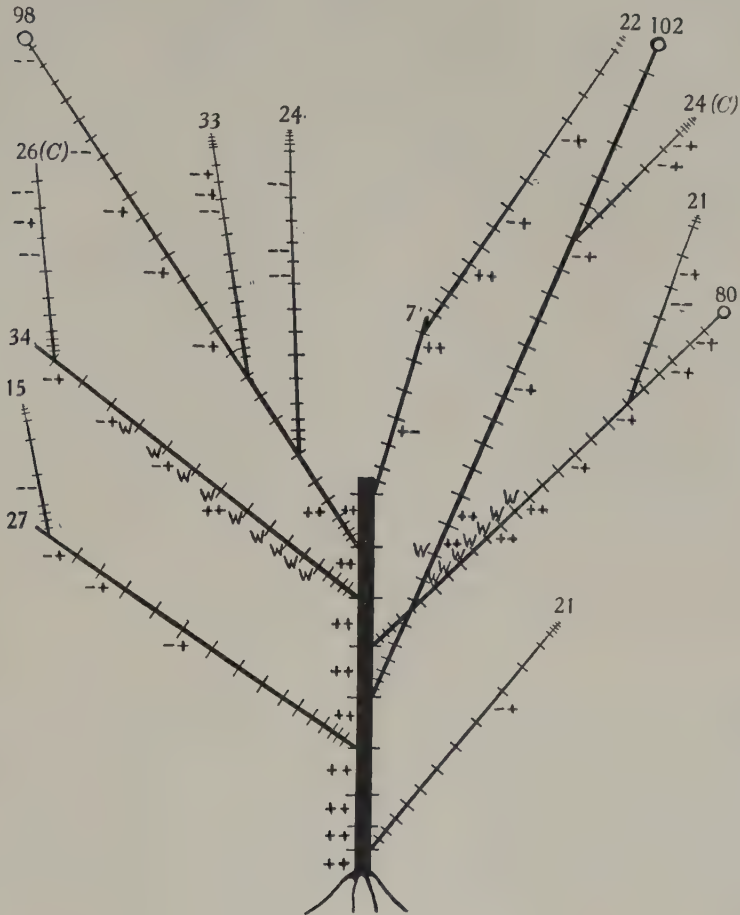


Text-fig. 5. Diagrammatic representation of a plant affected by *Verticillium* wilt as a result of wound inoculation, showing the distribution of vascular browning and of the fungus within the tissues. Explanation in text.

of the shoots were wilting. The nodes bearing these wilting leaves are indicated by a "W" alongside.

The length of each shoot (in cm.) is given by the number at its termination, the letter "C" in brackets indicating that the shoot was of the type selected for cuttings.

The examination of three other plants of the same variety, collected at the same time and showing the same slight symptoms, gave similar results.



Text-fig. 6. Diagrammatic representation of a plant naturally affected by *Verticillium* wilt, showing the distribution of the fungus within the tissues in relation to the distribution of wilting leaves and vascular browning. Explanation in text.

From this work it is concluded that *V. cinerescens* may be widespread within the tissues of plants showing only very early stages in the development of macroscopically visible internal and external symptoms of disease. The practical significance of this fact in relation to the carry-over of the disease by cuttings will be discussed in a later section.

A similar study has been made of the distribution of *Fusarium Dianthi* within the tissues of the plant in relation to external and internal disease symptoms. The three 1-year-old plants which were examined in detail were in an early phase of disease as judged from external appearance, viz. one side branch only showed the wilting symptoms. Here also, as for *Verticillium*, the fungus was isolated freely from branches which externally appeared perfectly sound. On the other hand, disorganisation of the tissues could be plainly seen extending through the various branches of the plant, and the fungus was in no case found to extend beyond the limits of the disorganised tissue.

The wide distribution of *V. cinerescens* within plants which show only slight and localised symptoms or which, as will appear later, show no symptoms at all, may be explained in a number of ways. The fungus may be continuous within the plant, growing from the collar upwards; alternatively the very minute spores may be carried up some distance in the transpiration stream and lodge at a point higher up where they begin to grow, the process being repeated. A third possibility is that the upper branches are merely contaminated by the lodgment of air-borne spores in the axils of the closely appressed leaves.

The localised appearance of fungal growth when an upper internode is plated out (Plate XXVII, fig. 7) as contrasted with the general growth from a piece of the collar (Plate XXVII, fig. 5) rather favours the view that distribution of the parasite is discontinuous. On the other hand, the observed effects might only mean that the fungal mycelium, while continuous, has a greater tendency to develop in some places than in others. That the source of infection is not air-borne spores which have lodged in leaf axils is suggested by the data of Table VIII. These refer to an examination of four plants of the variety Topsy, taken from the edge of a bare disease area, and showing no external symptoms of wilt. The symbols + and - in columns 2 and 3 refer as before to the presence or absence of vascular browning and of *V. cinerescens* respectively.

Table VIII.

Plant	In collar		No. of green internodes plated	No. of green internodes from which <i>V. cinerescens</i> were isolated
	Browning	<i>V. cinerescens</i>		
1	+	+	19	4
2	-	+	31	1
3	-	-	22	0
4	-	-	29	0

Though the experimental evidence is rather meagre, yet it definitely indicates a correlation between the amount of invasion of the collar and the frequency of occurrence of *Verticillium* in the upper parts of the plant. Invasion from below upwards and not surface contamination of the upper part is thus suggested. This view obtains support when it is remembered that *V. cinerescens* while capable of producing a dieback of the stopped shoots (v. p. 661) has not so far been found occurring naturally in this situation, so that presumably (at least in the nurseries where wilted plants are removed and destroyed at the first showing of symptoms) the spores of this fungus are not likely to be found in the atmosphere of the greenhouse.

The interest of this section lies in relation to the carry-over of disease in the cuttings. It is known that such a carry-over does take place (see later). The experiments described on p. 649, where it was shown that inoculation at unwounded nodes did not lead to disease, thus give further support to the view that the upper apparently healthy parts of the plant are in fact already invaded by the fungus. Whether that invasion is continuous or whether it may be partly in the form of isolated pockets of diseased tissue must remain an open question for the time being.

VII. THE INFLUENCE OF ENVIRONMENT ON THE INCIDENCE OF DISEASE.¹

A. MOISTURE CONTENT OF SOIL.

In view of the conflicting opinions amongst growers as to the influence of relative wetness or dryness of soil on the incidence of disease, and the apparent failure of Dowson's recommendation—to keep the beds on the dry side rather than the reverse—as a measure of control, the following experiment was performed.

Extensive bare areas at a commercial nursery on which, during the previous 2 years, every plant had succumbed to disease, were divided up into a series of plots by means of sheets of "Poilite" sunk to a depth of about 18 in. Each plot was from 3 to 4 ft. in length and about 3 ft. 6 in. wide (the width of the beds), and was separated from the plots on either side by buffer areas from 1 to 2 ft. in length.

¹ In this and in the succeeding section a number of the observations and experiments recorded were made before the composite nature of "stem rot" was known. The symptoms in every case were the same as those described by Dowson(11), and it appears probable that at least the great majority of plants were primarily affected by *Verticillium* wilt. This view is supported by the later demonstration of the predominance of this disease at all the nurseries concerned.

In addition to four control plots, which were watered in the normal manner, two plots were allocated to each of the following treatments:

A. The surface of the soil was given a concave camber, the centre being about 2 in. below the level at the sides of the bed. In this way a gradation from almost continuously dry surface soil at the sides to a continuously moist surface at the centre was maintained throughout the experiment.

B. The soil surface was given a convex camber, resulting in a similar gradation of moisture of the surface soil from the centre to the sides.

C. Only just sufficient water to maintain the turgidity of the plants was applied.

D. These plots were excessively watered, the soil never being allowed to become dry.

E. Two longitudinal rows of tiles were buried at a depth of about 9 in. below the surface, their ends being connected with upright chimneys that projected 1–2 in. above the surface of the soil. These plots were watered by pouring water down the chimneys, none being applied to the soil surface.

F. The plants were set out on the tops of ridges about 4 in. in height, and water was applied only to the furrows.

G. The plants were set out with only their roots embedded in soil, the collars being surrounded by a mulching layer of sand 2 in. in depth.

The object of treatments E, F and G was to test the theory, held by some growers, that moisture round the collars of the plants was conducive to infection. Treatment G was also designed so as to maintain continuously moist soil conditions round the roots, to check the theory held by some that an alternation of wet and dry soil conditions, by leading to the development of cracks in the collar, tended towards an increase in infection.

The treatments were distributed at random, except that no two plots with the same treatment were allowed to occur in the same bed, and, for more accurate comparison, each plot of treatment C adjoined one of treatment D.

At the beginning of May, each plot was planted up with six longitudinal rows of the variety Wivelsfield White, and for the first month was watered normally to allow the plants to become established. At the beginning of June the differential watering treatments were begun. From this time onwards until the end of the following March, weekly observations were made and every plant showing wilting symptoms was removed

and examined. A few of the plants here and there succumbed to wire-worm attack and to dieback, but these are disallowed in Table IX, which refers to plants showing symptoms of *Verticillium* wilt.

Table IX.

Plot	Treatment	No. of plants	Percentage of disease
A1 and 2	Concave camber: Highest rows (2)	24	75
	Intermediate rows (2)	24	75
	Lowest rows (2)	21	62
B1 and 2	Convex camber: Highest rows (2)	23	74
	Intermediate rows (2)	23	78
	Lowest rows (2)	23	65
C1	Subnormal watering	36	44
C2	"	35	80
D1	Excessive watering	36	44
D2	"	30	53
E1	Watered from below	32	50
E2	"	32	55
F1	Ridged	34	91
F2	"	32	72
G1	Sand layer	35	57
G2	"	33	48
H1	Controls, normally watered	35	0
H2	" "	30	60
H3	" "	34	50
H4	" "	35	49

The relative significance of these figures is not clear, but at any rate it is plain that under all the variety of moisture conditions set up very serious disease resulted. The complete absence of disease in one of the control plots (H1) may be noted. Whether this is connected with some soil factor or what is the explanation is quite unknown.

B. TEMPERATURE.

As shown in a previous section, the normal appearance of disease at commercial nurseries is as follows. During the first year after planting only comparatively few scattered plants are observed to be diseased, and but little spread from these foci is apparent until the spring and summer of the second year. A rapid extension of disease areas then takes place, followed by a diminution of the rate with the advent of the following autumn and winter, which increases again during the warmer months of the third year. These observations might be taken to indicate that comparatively low temperatures are unfavourable to infection. Such an assumption does not, however, take account of the length of the incubation period.

In a disease of this nature it is reasonable to suppose that the turgidity of an affected plant at any given time will depend on two

factors; the extent to which the vascular system is disorganised as a result of fungal invasion, and the demand made on the vascular system by the transpirational needs of the plant. Each of these factors will in turn be governed by temperature conditions, the former in so far as temperature influences the rate of development of the fungus within the tissues, and the latter inasmuch as an increase of temperature means an increase in the transpiration rate. It would therefore appear probable that the incubation period of the disease is longer at comparatively low temperatures, and some evidence in support of this has already been obtained (see pp. 651-2).

In experimental work the shortest incubation period yet found was, in the case of wound inoculation 7 weeks, and for the soil contamination method 10 weeks. Corresponding figures for an experiment in which plants were inoculated in January were 10 and 13 weeks respectively. It should be noted that, even in this latter experiment, no symptoms of disease appeared in any plant until the latter half of March, a time at which somewhat higher temperatures are already beginning to prevail in the greenhouse. It is therefore not unlikely that if similar plants had been inoculated in October an even longer incubation period would have been found.

In the light of the foregoing argument it seems not improbable that the appearance of a comparatively large number of wilted plants in the warmer months of the year may be due to a retardation of the development of symptoms in the cooler months, rather than to any favourable influence of relatively high temperatures on infection. That considerable infection can occur during the autumn and winter is indicated by the results of the following experiment. A length of bed on which every plant had succumbed to disease was divided by cross-walls into four plots. From one of these the old top soil was removed and fresh soil that had never before grown carnations was put in its place. In the others the old top soil was retained. Each plot was planted up with variety Mrs A. J. Cobb and weekly observations were then made, every wilted plant being removed for examination and found to be affected by *Verticillium* wilt. The results are given in Table X.

Table X.

Treat- ment	No. of plants (original)	No. of plants showing symptoms of <i>Verticillium</i> wilt by					
		18. viii. 33	21. ix. 33	22. xii. 33	26. iii. 34	28. v. 34	22. vi. 34
Top soil replaced	32	—	—	—	—	—	1
Top soil retained	96	2	18	35	50	95	96

From these figures it is seen that, allowing that the minimum incubation period is about 2 months, every plant but one of those planted in the old top soil had become infected by the end of ~~March~~, and the majority of them during the months of autumn and winter. *May!*

From the foregoing theoretical considerations and experimental data, the normal incidence of disease at commercial nurseries may be explained as follows. The rarity of disease between the time of planting and the following spring is due, at least at first, to non-contact of the plants with contamination. Such sporadic wilting as does occur arises from the plants being infected as cuttings (*vide* next section). Later, during the first autumn and winter, considerable infection may take place by the growth of roots into contaminated areas of the old subsoil, and also possibly by the spread of the causal organism from foci set up by those plants, infected as cuttings, that have succumbed to the disease. The fact that but little indication of this is given during the cold period may be explained by assuming that in only comparatively few of the infected plants is there a sufficient development of the fungus in the vascular system to cause the appearance of external symptoms. With the advent of warm days in spring, the presence of these infected plants would be shown up by the development of wilting symptoms, brought about as a result of the increased transpirational demands on the disorganised vascular system. This would explain the frequently observed sudden rapid development of symptoms in large numbers of plants at this time. From now onwards the spread of infection may proceed at an approximately uniform rate, the appearance of a decreased rate in autumn and winter followed by an increase in the warmer months being due not to an influence of temperature on infection but to its influence on the length of the incubation period. In this connection it may be remembered that *Verticillium cinerescens* behaves in culture as a "low temperature" organism.

While the writer favours the interpretation just put forward, he is aware that nothing short of experiments carried out under properly controlled conditions of temperature will settle the question.

VIII. A DISCUSSION OF POSSIBLE CONTROL MEASURES.

A. BY THE CHECKING OF THE SPREAD OF DISEASE¹ AREAS.

In the early stages of this research, attempts were made to check the further extension of areas of disease in commercial nurseries by the use of chemicals and by placing mechanical barriers across the bed at various

¹ See footnote on p. 667.

distances from the visible limits of disease. These gave no permanent success.¹

The fundamental difficulty in attempting such methods lies in the lack of knowledge regarding the incubation period of the disease in different varieties of the host plant under a range of environmental conditions and at various stages of growth. In the light of the experience gained it seems probable that the putting down of chemical or mechanical barriers has hitherto proved of no avail, inasmuch as the parasite may have already spread, from foci set up by the planting of already infected plants or by other means, far beyond the limits of the visibly contaminated areas. It is also possible that the pathogenic organism may have been present from the start in extensive areas of the subsoil, but had only been able to manifest its parasitic activity in comparatively restricted areas by the time that the barriers were laid down. In any case, whatever be the source of infection, the method does not appear to hold any hopeful prospects.

B. BY THE DESTRUCTION OF THE ORGANISMS OF DISEASE IN THE SOIL.

(1) *By chemical methods.*

Concurrently with the experiment on the influence of soil moisture conditions on the incidence of disease described in section VIIA, eighteen contaminated soil plots were subjected to nine different chemical treatments in duplicate as follows:

K. Watered with Cheshunt compound— $6\frac{1}{2}$ gallons per sq. yd.

L. Watered with formalin (1 : 50), and then left covered with sacks soaked in the solution for 2 days—5 gallons per sq. yd.

M. Watered with mercuric chloride solution (1 : 1000)— $1\frac{1}{2}$ gallons per sq. yd.

N. Watered with Uspulun solution (1 : 200)— $1\frac{1}{2}$ gallons per sq. yd.

O. Top-dressed with Uspulun powder—4 oz. per sq. yd.—which was then thoroughly incorporated with the top 6–8 in. of soil.

P. The soil was similarly treated with quicklime, at the rate of $4\frac{1}{2}$ lb. per sq. yd.

Q. As a control to any possible sterilising effect due to the heat produced by treatment P, the soil was similarly treated with slaked lime at the rate of 6 lb. per sq. yd., thus giving approximately equal Ca.

¹ The following methods were employed: disease areas watered with Cheshunt compound; 6-in. deep trenches beyond the visible limits of disease areas filled with 0.5 per cent. Uspulun solution or with quicklime; disease areas partitioned off from the rest of the bed by sheets of "Poilite" sunk to a depth of 6 in. (the greatest depth practically possible owing to the wiring).

R. Watered with sulphuric acid—1/50th pint in $1\frac{1}{2}$ pints water per sq. ft.

S. As in R, but followed by the application of theoretically just sufficient slaked lime to neutralise the acid applied.

All of these treatments were carried out a fortnight before planting. The results of this experiment, computed in the same way as those of the concurrent experiment described in section VIIA, are given in Table XI. The four control plots were common to both experiments.

Table XI.
Var. Wivelsfield White.

Plot	Treatment	No. of plants	Percentage of disease after 11 months
K1	Cheshunt compound	36	75
K2	"	30	20
L1	Formalin	35	0
L2	"	34	24
M1	Mercuric chloride	36	81
M2	"	30	77
N1	Uspulun (solution)	33	36
N2	"	35	29
O1	Uspulun (powder)	31	16
O2	"	36	14
P1	Quicklime	36	16
P2	"	34	44
Q1	Slaked lime	39	70
Q2	"	35	57
R1	Sulphuric acid	36	75
R2	"	29	58
S1	Sulphuric acid + slaked lime	34	47
S2	" "	28	25
H1	Controls	35	0
H2	"	30	60
H3	"	34	50
H4	"	35	49

As is clear from the behaviour of duplicate plots, there is a very pronounced experimental error involved, and the figures must therefore be interpreted very conservatively. Clearly the treatments with mercuric chloride, slaked lime, and sulphuric acid, and probably also with Cheshunt compound and quicklime, were quite ineffective. The four plots treated with Uspulun showed less disease, but whether this represents a significant result or not is immaterial as there was pronounced stunting of the plants.

The formalin-treated plots gave a somewhat more promising result. No significance can be attached to the entire lack of disease in plot L1, since this plot was adjacent to the control plot H1 (cf. p. 669). On

plot L2, however, it was observed that no disease appeared until after 5 months from planting and only two plants were affected after 9 months. The incidence of disease on this plot was therefore of the same type as occurs when beds in which disease has appeared are replanted after replacement of the old top soil. On the other hand, all the other treated plots showed a development of disease similar to that resulting from the planting up of disease areas with the top soil retained (see Table X). It may also be added that the formalin treatment caused a marked stimulation in growth of the plants.

The above data therefore indicate that, of the nine treatments tested, only formalin has apparently had any sterilising effect, and it seems possible that, under certain conditions to be indicated later, this treatment may prove to be of some value.

(2) *By steam sterilisation.*

Sterilisation of the beds by steam has been practised on a number of nurseries, with varying results. The writer has, however, not yet seen what can be described as a satisfactory measure of control by this method, and on occasion it even appears to have resulted in greater losses than before.

Failure of steaming, or of any other method of sterilisation, as a means of controlling the disease, may be ascribed to either or both of the following factors:

(a) failure to destroy all contamination in every part of the soil with which the roots may come in contact, or from which the parasite may spread to the host plant, and

(b) the introduction of disease organisms into the soil after the treatment.

The way in which these factors may operate to negative any sterilising treatment will be indicated in the following subsection, in which the major sources of infection of the new crop are considered.

C. BY THE ELIMINATION OF POSSIBLE SOURCES OF INFECTION.

(1) *The top soil and side walls.*

Both in experimental work and in commercial practice, the planting up of areas on which the incidence of *Verticillium* wilt has been high, without replacement or chemical treatment of the top soil, has invariably in the writer's experience resulted in a very severe loss of crop within a year of planting. In contrast to this, the replacement of the old top soil by soil that has never before carried carnations has always, so far

as the writer is aware, been followed by the appearance of disease in only comparatively few scattered plants during the first year. As shown later, it is probable that at least some of the plants that show symptoms of disease during this period were actually infected as cuttings, and it would therefore appear that the fresh soil may be considered to be relatively, if not entirely, free from contamination by *V. cinerescens*.

The host range of this fungus is as yet unknown, except that it has been recorded as causing a wilt of China asters in Germany (3).

It may therefore be concluded that, where *Verticillium* wilt has occurred in the previous crop, it is essential in order to avoid a serious recurrence of the trouble in the first year to replace the old top soil by soil that has never before grown carnations. Whether soil that has carried any other crop should also be excluded must remain unknown until the host range of the causal organism is determined.

The wood, brick or concrete of the side walls of a bed which has carried a diseased crop is obviously to be looked on with suspicion, and if the new soil is not to be sterilised *in situ*, the side walls should be washed with creosote. This treatment has been tested at a commercial nursery and found to cause no ill-effects to the plants.

(2) *The subsoil.*

The role of the subsoil in carrying over infection from one crop to the next may be illustrated by the following example.

Over a bed in which extensive disease had appeared in the previous crop a raised bed was built, the brick floor being supported by cross-rows of bricks laid on the old subsoil. By this means it was hoped that penetration of the roots into the latter would be prevented. The bed was filled with soil that had never before grown carnations, and was planted up with a stock of variety Mrs A. J. Cobb which was, as shown by the freedom of the crop from disease during the first year, free from infection. In the spring of the second year, however, a number of plants developed the symptoms of *Verticillium* wilt, and the disease spread rapidly until by the end of the summer extensive bare areas were to be seen. It was found when the bed was dismantled that, contrary to plan, the roots had penetrated in abundance into the old subsoil. In the absence of any other likely source of infection, it is probable that the incidence of disease was due to subsoil contamination.

Sterilisation by heat or by chemicals of a sufficient depth of the subsoil may be difficult if not impracticable. Failing that it will be necessary to ensure that the roots of the new crop are prevented from

penetrating the old subsoil. This problem has been met by a number of growers by the adoption of raised beds of various types such as are described on p. 635. So far these measures have met with considerable success, which is becoming greater as the grading up of the stock, to be described below, is being proceeded with.

(3) *The stock.*

The occurrence of *Verticillium* wilt among the young plants, both in pots and after they have been set out in the beds, in spite of sterilisation of the potting soil by baking and the use of clean pots and stakes, has led to a general conviction amongst growers that the disease may be carried over to the new beds by the cuttings. With the object of avoiding this source of infection, a number of raised beds were built in a house in which carnations had never before been grown. They were filled with soil that had never carried carnations, and were then planted up with stocks of the different varieties under cultivation at the nursery. These plants were then continuously stopped throughout the year, the object being the production of as many shoots, suitable for the making of cuttings, as possible. In the following winter cuttings were taken from this house in the usual way, the neighbourhood of the very few gaps where one or two plants had succumbed to the disease being strictly avoided.

The number of cuttings provided by this special house proved insufficient to meet the demands of the planting programme for the following spring, and to make up the deficiency a number were taken from the flowering houses, the neighbourhood of disease areas being as far as possible avoided. These were treated in exactly the same way as those from the special house, the same sample of baked potting soil being used for each.

The difference in the percentage of diseased plants between the stocks raised by the two different methods was very striking, that in the stock raised by the special method being estimated at less than 0.1 per cent., while more than 10 per cent. of the plants raised from cuttings taken from the flowering houses succumbed to the disease while still in the "60" pots. The only reasonable explanation of this difference appeared to be that a greater proportion of the cuttings taken from the flowering houses were infected before removal from the parent plants, a conclusion which is supported by the results of the following experiments.

At intervals from November to February, samples of cuttings from *apparently healthy plants*, of various ages and varieties and at different

distances from visibly contaminated areas in the flowering houses, were examined in each of the following ways:

(a) By plating out a number of internodes of each in the manner described in section VI.

(b) By growing the cuttings in the ordinary way. These were accorded exactly the same treatment as those taken from a special house, of the type described above, which was apparently almost entirely free from disease.

The results obtained from these experiments are collected together in Tables XII and XIII.

Table XII.

Variety	Age of parent plants (approx.) years	Position of parent in relation to areas of visible disease	No. of cuttings		% infected cuttings (approx.)
			Examined by plating method	Found to be infected with <i>Verticillium cinerescens</i>	
Topsy	2	Adjacent	70	9	13
Spectrum	2	"	164	11	7
"	2	As far removed as possible	181	0	0
Mrs A. J. Cobb	1	Adjacent	124	1	1

Table XIII.

Variety	Age of parent plants (approx.) years	No. of cuttings	No. showing symptoms of <i>Verticillium</i> wilt within in weeks				
			7	17	24	27	28
Topsy	2	30	8	8	18	18	22
Spectrum	2	60	2	4	4	5	7
Mrs A. J. Cobb	1	24	0	0	4	6	9

There was thus very significant contamination of the cuttings, whether tested by the method of plating or by striking the cuttings. The latter method gave in the long run considerably higher figures, which may mean either that the plating method gives an underestimate of the amount of contamination or that the fungus spreads appreciably from one cutting to another in the sand of the propagating bed. It will be noticed that, for some reason or other, the symptoms are slow in appearing in some of the cuttings, so that if they had been planted out in beds at the usual time the practical result would have been the re-establishment of new centres of infection in the flowering houses. It is certain that in commercial practice reinfestation of the beds commonly occurs in this way.

By way of contrast to the figures shown in Tables XII and XIII, it may be stated that there was less than 0.1 per cent. of diseased plants

in the stock which was being raised at the same time from cuttings taken from the special house.

Up to the present the carry-over of disease by cuttings has only been proved experimentally when these were taken from the border of a disease area. That plants growing some distance away from such an area are less likely to be infected is what one would expect. It must be pointed out, however, that the number of plants so far examined in this respect is not large, so that it is not safe to assume that a proportion of infected cuttings may not be picked up even when the nurseryman strictly avoids the neighbourhood of disease areas.¹ In practice this may be difficult to arrange in certain beds and with certain varieties where the disease occurs sporadically throughout the whole stand, *i.e.* if the necessary number of cuttings is to be obtained.

A further point is whether infection of cuttings is more likely to occur when they are taken from 1-year-old or from 2-year-old plants. The data of Table XII suggest that 1-year-old plants are less likely to give infected cuttings, but it will be noticed that, so far, no strict comparison has been made between 1-year-old and 2-year-old plants of the same variety. It may be that 1-year-old plants are preferable inasmuch as the fungus may not have had time to invade the upper branches. On the other hand, it is not impossible that, in taking cuttings from a 1-year-old stand, one is using plants which are destined to die some months later and which are already permeated by the fungus. If such is the case, it might be advisable to take the cuttings from 2-year-old plants, since the main disease areas would by that time have shown themselves. More detailed work on this point is necessary before a definite answer can be given.

The risk of carrying over the disease by infected cuttings is a very serious one, and unless it can be dealt with the new control measures based upon steam sterilisation of the beds and the isolation of the latter from the subsoil will be more or less defeated. The slight amount of evidence as yet available suggests that 1-year-old plants only should be used for the propagation of cuttings, but as hinted above this method may prove to be illusory. An alternative plan would be to try to discover some way by which infected cuttings would develop external symptoms of the disease while they were still in the pots. Possibly a somewhat high temperature in the propagating house combined with reduced watering of the soil, if practicable in other respects, would help in the attainment of this object. Failing that, it might be suggested that the cuttings should

¹ Practical experience does in fact suggest that some of the cuttings are infected when such precautions are taken.

be kept in the pots under ordinary conditions for a time sufficient to allow the diseased individuals to be recognised. The last measure would not be practicable on a large scale, but might be used to furnish a nucleus of healthy plants from which a clean stock could in time be developed.

D. BY THE DEVELOPMENT OF NEW RESISTANT VARIETIES, OR OF
RESISTANT STRAINS OF EXISTING VARIETIES.

Although from time to time claims have been made for a number of different varieties that they show an appreciable degree of resistance, the writer has not as yet seen any variety, of sufficient merit to be widely grown on a commercial scale, that has consistently remained reasonably free from disease. It would, however, be strange if, among the thousands of seedlings raised every year by growers who specialise in the development of new varieties, there were not some, of little commercial value perhaps in themselves but suitable as parents for further breeding, that were not more resistant than the varieties now cultivated. It is therefore suggested that bare disease areas in the beds might be usefully employed for testing numbers of such seedlings. From any that survived, cuttings should be taken, and the progeny again tested either by the direct inoculation method or by planting in contaminated soil. If by such means any real measure of resistance in the progeny of any seedling is clearly shown up, breeding in the hope of raising new resistant varieties can be undertaken with at any rate a somewhat greater chance of success than at present exists.

On a number of occasions there have been observed apparently healthy plants standing alone in extensive bare disease areas. From a number of such plants cuttings have been taken, and the progeny of each tested against the ordinary stock of the same varieties by planting in contaminated areas of beds. In no case has any greater degree of resistance been found in the progeny of such plants. This method should, however, be persevered with, since if there should be found a relatively resistant sport of any variety, there would be to hand not only a parent from which a stock of a resistant strain of the existing commercially valuable variety could be built up, but also a starting-point for attempts to develop by breeding new resistant varieties.

IX. SUMMARY.

1. An account is given of the occurrence of disease, known by growers generally as "stem rot" and "dieback", among Perpetual Flowering Carnations under commercial culture in England.

2. By the plating out of collars of diseased plants, the following four fungi have been more or less frequently isolated: *Verticillium cinerescens* Wr.; *Fusarium culmorum* (W. G. Smith) Sacc.; *F. herbarum* (Corda) Fries.; and a fungus provisionally identified as *F. Dianthi* Prill. et Del. When isolations were made from the inner parts of stems, viz. from xylem and pith only, there was a great reduction in the frequency of isolation of *Fusarium culmorum* and *F. herbarum*.

3. These four fungi have been inoculated into plants at various stages of growth under normal cultural conditions. From the results, which are summarised on pp. 653-4, it is concluded that "stem rot" is a complex of three symptomatically and etiologically distinct diseases, as follow:

- (a) *Verticillium* wilt, caused by *V. cinerescens*.
- (b) *Fusarium* wilt, caused by *F. Dianthi* (provisional identification).
- (c) Stem rot, caused by *F. culmorum*, *F. herbarum*, and possibly other *Fusarium* spp.

A diagnostic key to these diseases is given on p. 654.

4. Dieback, the symptoms of which are described on p. 660, appears to be caused mainly by *F. culmorum*. In one experiment inoculation by *Verticillium cinerescens* has also resulted in the development of a dieback type of disease. To what extent this may occur naturally is unknown.

5. The relative economic importance of the above diseases is discussed (pp. 662-3).

6. Morphological and cultural features of the causal organisms are described, and experimental data on the influence of temperature on their growth are given.

7. In examination of the distribution of the wilt organisms in plants showing only very early signs of being diseased, *V. cinerescens* has been isolated from parts of shoots well beyond the limits of any macroscopically visible internal or external symptoms. The possible means by which these parts become infected are discussed. *Fusarium Dianthi* has also been isolated in similar circumstances, but has not as yet been found to occur beyond the limits of visible vascular alteration.

8. The influence of soil moisture and temperature conditions on the incidence of *Verticillium* wilt is discussed.

9. It is shown that infection of a fresh crop by *V. cinerescens* may occur (a) as a result of the presence of contamination left by the preceding crop in the top soil and subsoil, and (b) by the introduction into the beds of a number of apparently healthy but actually infected plants,

developed from cuttings which were themselves already infected before removal from the apparently healthy parent plants.

It is pointed out that until this latter source of recontamination of the beds can with certainty be avoided, control methods based upon isolation and sterilisation of the beds will fail to eradicate the disease.

In the absence of any consistently resistant variety in general cultivation, it is recommended that a search for resistance be made amongst the numerous seedlings raised by specialists in the development of new varieties, and that the possibility of the occurrence of resistant sports in existing varieties be not overlooked.

The above work was carried out during the author's tenure of an Agricultural Research Scholarship. Subsequently its continuation for a fourth year was made possible by means of a grant from a group of carnation growers. This latter scheme was organised by the British Flower Marketing Association, to which and in particular to its Secretary, Mr A. G. Forsyth, the author wishes to express his thanks. For the provision of facilities for carrying out experiments under commercial conditions, special thanks are due to Messrs Dudley Page of Hanworth and Messrs Dutton and Son of Iver. As regards the scientific aspect of the work, the author is indebted to Dr H. W. Wollenweber for assistance and advice during the course of three months spent in his laboratory and also for the identification of organisms which were sent to him from time to time. Finally thanks are due to Prof. W. Brown of the Imperial College of Science and Technology, South Kensington, who first brought the problem to the author's notice and under whose general direction the investigation was carried out.

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Fig. 1.



Fig. 2.

WICKENS.—WILT, STEM ROT, AND DIEBACK OF THE PERPETUAL FLOWERING
CARNATION (pp. 630-683).



Fig. 3.

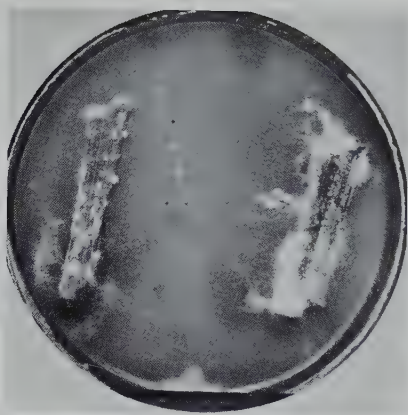


Fig. 4.

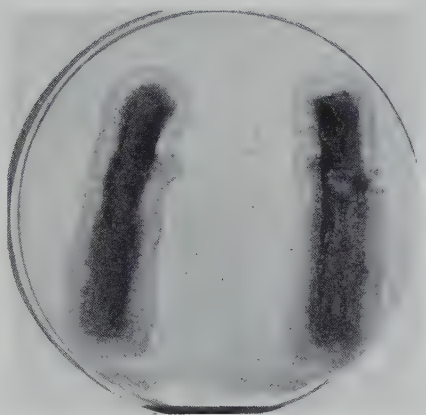


Fig. 5.

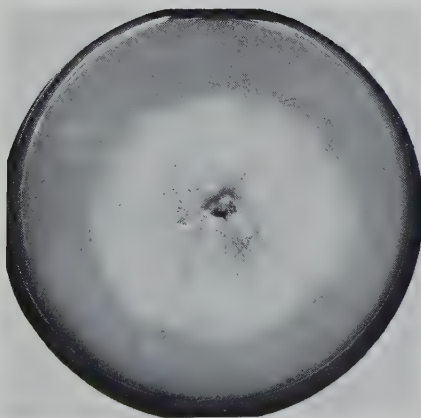


Fig. 6.



Fig. 7.

WICKENS.—WILT, STEM ROT, AND DIEBACK OF THE PERPETUAL FLOWERING
CARNATION (pp. 630-683).

EXPLANATION OF PLATES XXVI AND XXVII.

PLATE XXVI.

- Fig. 1. Stem rot caused by *Fusarium culmorum*. Left and centre: plants inoculated through nodal wounds at time of transference from sand to small pots; on left, slight basal rot and localised death of leaves—no further symptoms developed; centre, complete basal rot causing death of the plant. Right: control plant.
- Fig. 2. Wilt caused by *Verticillium cinerescens*. Left: control plant. Centre and right: plants inoculated as above; centre, showing unilateral leaf wilting and a striking curvature towards the affected side, and on right, showing unilateral leaf wilting and "kinking" at a node, the upper part of the stem being inclined towards the affected side.

PLATE XXVII.

Typical growths of the causal organisms of stem rot and wilt on isolation plates.

- Fig. 3. Culture of *Fusarium culmorum* (left) showing extensive lobular growth contrasted with growth of *Verticillium cinerescens* (right) on the same plate—from under side.
- Fig. 4. *Fusarium Dianthi*, showing sclerotial bodies developing on the stems and on the surface of and within the medium.
- Fig. 5. *Verticillium cinerescens* growing out from diseased collars of carnation plants.
- Fig. 6. The same on oatmeal agar, showing dark sporodochium-like aggregates of penicilli.
- Fig. 7. The same from an internode of a green shoot showing no symptoms of disease. Note localised development from the lower end, in contrast to the uniform growth from the collar illustrated in Fig. 5.

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FURTHER EXPERIMENTS ON THE *FUSARIUM* BULB ROT OF *NARCISSUS*

BY LILIAN E. HAWKER, M.Sc., PH.D.

(From the Department of Mycology and Plant Pathology,
Imperial College of Science and Technology, London.)

(With 2 Text-figures.)

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I. INTRODUCTORY.

IN an earlier publication Gregory (2) described observations and experiments on a disease of *Narcissus*, in which rotting of the bulbs takes place, chiefly under conditions of storage, and which is independent of eelworm invasion. He showed that the organism chiefly concerned was *Fusarium bulbigenum* Cke and Mass., so that the disease is referred to as the *Fusarium* bulb-rot of *Narcissus*. The mode of entry of the parasite into the bulb was studied and a preliminary examination of possible methods of disease control was carried out.

The present paper is an account of work which is essentially a continuation along certain lines of the foregoing. Particular attention has been paid to the question of the mode of entry of the parasite and to methods of disease control. Experimental work is still in progress, but certain results are sufficiently clear to warrant publication.

II. MODE OF ENTRY OF THE FUNGUS INTO THE BULB.

Gregory's statements, that the *Fusarium* is unable to penetrate the intact surface of the bulb scale, whereas it readily destroys the tissue of certain varieties when placed in a superficial wound, and that, in stocks naturally infected, the disease occurs more frequently in "double-nosed"

than in "round" bulbs, have been confirmed. As regards penetration *via* the root, Gregory's experiments were not comprehensive and gave negative results. He found, however, that by merely immersing bulbs for some time in a warm or cold suspension of *Fusarium* spores and subsequently storing them at a rather high temperature, a considerable percentage rotted.

Further work has been carried out along these two lines and the results are here described.

(i) *Infection of roots of Narcissus by Fusarium bulbigenum.*

Gregory carried out a number of root-inoculation experiments but was not able to induce the fungus to enter the bulb through the roots. In one case, where bulbs of variety Emperor were dipped in a suspension of *Fusarium* spores and stored for a few days at 10–12°C. before being transferred to higher temperatures, the roots were parasitised but the bulbs remained sound.

He also failed to induce rotting of either roots or bulbs by allowing the roots to dip into a spore suspension; by inoculating the roots, in July, with pieces of agar culture; by inoculating the roots of potted bulbs by means of pieces of agar culture pushed down glass tubes, which had been previously inserted below the bulbs; or by planting bulbs in the open over mycelium of the fungus or pieces of parasitised bulb scales.

The writer's experiments were carried out with a stock of 250 bulbs of the variety Victoria and with smaller batches of varieties Henry Irving, Seagull, Golden Spur and Spring Glory, during November and December.

Rooting of the two last-named varieties was brought about by soaking the bulbs for 3 hours in cold water followed by storage in a moist dish at laboratory temperature, about 15°C. This method was not completely satisfactory, since moulds were liable to develop on the surface of the bulbs or roots during the storage period. In the case of the three other varieties, the roots were caused to emerge by planting the bulbs in the usual way in moist bulb fibre. About 3 weeks after planting, these bulbs were taken from the boxes. The roots were washed to remove adhering particles of fibre and carefully dried with blotting paper before inoculation. This method, while satisfactory as regards the production of healthy roots, had the disadvantage that the latter were easily damaged in the process of cleaning. All bulbs with visibly damaged roots were discarded. By whichever method rooting was effected the results of inoculation were the same.

The method of inoculation was to dip the roots into a suspension of *Fusarium* spores, care being taken to avoid wetting the bases of the bulbs. Where the aim was to test whether infection could take place in the soil, the bulbs were inoculated as above or were planted so that their roots would later come in contact with portions of agar culture of *F. bulbigenum* or with pieces of diseased bulb scale from which the *Fusarium* had been isolated.

In one experiment, which may be described in detail as an illustration, twenty bulbs of variety Victoria, the roots of which had been dipped in a suspension of *Fusarium* spores, were stored, one half of them in closed moist vessels at 27°C. and the other half similarly in an unheated shed, the temperature of which varied from 0 to 17°C. during the experiment. An equal number of control bulbs, the roots of which had been dipped into sterile water, were stored under similar conditions. After a few days the tips of the roots of the inoculated bulbs at 27°C. had become reddish brown (the colour which is typical of *Fusarium* disease) and attack was progressing up the roots towards the base of the bulb. Ten days after inoculation rotting of the roots was complete and had advanced up the bulb scales for a distance of about 1 cm. from the base. The other batches were sound, with the exception of one of the inoculated lot in the cold shed, where, although the bulb was sound, the tips of the roots were discoloured and rotting. Later all the inoculated bulbs at 27°C. rotted completely and a few of each of the other batches were slightly rotted, suggesting that some of this stock must have been naturally infected before inoculation. These results are set out in Table I.

Table I.

(Number of bulbs in each batch=10.)

Treatment of roots	Temperature of storage °C.	No. of bulbs rotting after 10 days	No. of bulbs rotting after 6 weeks
Dipped in spore suspension	27	10	10
" sterile water	27	0	2
" spore suspension	0-17	0	2
" sterile water	0-17	0	1

The results of the various experiments in which inoculated bulbs were subsequently stored are assembled in Table II.

The main conclusion to be drawn from Table I is that inoculation of the roots followed by storage at temperatures of 20, 27 or 30°C. led, with the exception of one of the varieties used, to the loss of a high proportion of the bulbs. When storage was at a lower temperature (0-17°C. and 10-16°C. in the table), the mortality of inoculated bulbs

was much less, and in the majority of varieties there was no loss. While successful infection is thus largely dependent upon a suitably high storage temperature, the conditions of experiment were not sufficiently exact to indicate the minimum temperature for attack.

Table II.

Variety	Treatment	Storage temperature °C.	No. of bulbs used	No. with roots and bulbs rotted	No. with roots only rotted
Victoria	Roots inoculated	27	10	10	0
	"	20	5	5	0
	"	0-17	15	4	1
	Not inoculated	27	10	2	0
	"	20	5	0	0
	"	0-17	15	1	0
Henry Irving	Roots inoculated	27	10	7	3
	"	0-17	10	0	0
	Not inoculated	27	10	0	0
	"	0-17	10	0	0
Seagull	Roots inoculated	27	10	0	10
	Not inoculated	27	10	0	0
Spring Glory	Roots inoculated	30	10	9	0
	"	10-16	10	0	0
	Not inoculated	30	10	0	0
	"	10-16	10	0	0
Golden Spur	Roots inoculated	30	10	9	1
	"	10-16	10	0	0
	Not inoculated	30	10	0	0
	"	10-16	10	0	0
Total	Roots inoculated	20-30	55	40	14
	"	0-17	45	4	1
	Not inoculated	20-30	55	2	0
	"	0-17	45	1	0

In all varieties used except Victoria, the uninoculated controls remained healthy at whatever temperature they were stored. They were therefore presumably free from latent natural infection. The presence of a certain amount of natural infection in the stock of Victoria is illustrated in Table I and has already been noticed.

The variety Seagull showed exceptional behaviour in one respect. Whereas in the other varieties, rotting of the roots led almost invariably to rotting of the bulbs, the process was arrested at the junction of root and bulb in the case of Seagull. This behaviour is comparable with that recorded by Gregory for the variety Emperor. Both these varieties are known to be somewhat resistant to the disease, and the effect just described may be an illustration of this feature.

Ten of the Victoria bulbs, listed in Table II, were inoculated by allowing their roots to dip into a suspension of *Fusarium* spores during the whole period of storage, the roots of control bulbs being allowed to dip into sterile water. Half these bulbs were stored at 20°C. and half

in the cool shed (0–17°C.). Within a few days a web of hyphae was formed round the tips of the roots of the inoculated bulbs stored at 20°C. The root tips then developed the typical reddish brown colour and the roots slowly rotted from the tip upwards. The outer bulb scales began to rot about 1 week after the experiment was set up and later the bulbs rotted completely. The uninoculated controls remained sound and the roots continued to grow during the experiment. Some of the roots of the inoculated batch in the cool shed rotted after a time and two of these bulbs were slightly rotted after 6 weeks. Gregory did not obtain any rotting of the roots or bulbs of the susceptible variety *Madame de Graaf* in a similar test. It is suggested that the positive result obtained in the present case may have been due to the precaution which was taken of shaking the flask each day, whereby the spores tended to remain in suspension.

The fact that, in these experiments, the tips of the roots rotted first, suggests the possibility that the tip is the most susceptible part of the root. The conditions of experiment were such, however, that an uneven distribution of the spores over the root surface was possible, and further detailed work would be necessary to determine whether the older parts of the roots are susceptible. If one postulates such a localisation of susceptibility of the roots one can readily explain Gregory's failure to induce attack in cases where the roots were inoculated with pieces of agar culture, since it would then be possible that the inoculum never came into contact with a susceptible part of the root.

The dependence of infection upon a somewhat high temperature also probably explains some of the negative results recorded by Gregory, *e.g.* when inoculated bulbs were planted outside in the late autumn.

In all the experiments described above the bulbs were stored after inoculation, but tests were also carried out in which the inoculated bulbs were planted in pots of sterilised soil or in field plots. The potted bulbs were placed either in a warm greenhouse, where the temperature varied around 20°C., or in a cool shed (0–17°C.), or at laboratory temperature (10–16°C.).

The results of all tests in which the bulbs were afterwards planted in soil are collected in Table III.

The results given in Table III are similar to those obtained with stored bulbs. Out of twenty bulbs of variety *Victoria*, which had been inoculated by dipping the roots in a suspension of *Fusarium* spores and had then been potted and kept in the greenhouse for 4 months, twelve were badly rotted, seven slightly so and one bulb was sound although

the roots were rotted. Bulbs treated similarly but stored in the cool shed were sound and the roots were white and healthy. Control bulbs, the roots of which had been dipped in sterile water before planting, were sound, even when kept in the greenhouse.

Table III.

Variety	Treatment	Storage conditions	No. of bulbs used	No. with roots and bulbs rotted	No. with roots only rotted
Victoria	Roots inoculated	Greenhouse	30	29	1
"	"	Cool shed	20	0	0
"	"	Planted in open	20	0	0
"	Not inoculated	Greenhouse	20	0	0
"	"	Cool shed	20	0	0
"	"	Planted in open	20	0	0
Spring Glory	Roots inoculated	Laboratory	10	0	0
Golden Spur	Roots inoculated	Laboratory	10	0	0
Total	Roots inoculated	Warm	30	29	1
	"	Cool	60	0	0
	Not inoculated	—	60	0	0

A similar high proportion of attack was obtained by planting bulbs of variety Victoria, the roots of which were already 1–5 cm. long, above pieces of agar culture of *F. bulbigenum* or above scales of diseased bulbs, from which the fungus had been isolated, and keeping them in the warm greenhouse.

Bulbs of varieties Spring Glory and Golden Spur, the roots of which were inoculated by being dipped in a spore suspension and which were planted in soil and kept in the laboratory (average temperature 15°C.) were sound when examined 4 months after inoculation. The failure of the fungus to attack was presumably due to the relatively low temperature.

Twenty bulbs of variety Victoria, the roots of which had been dipped in a spore suspension, together with control bulbs, the roots of which had been dipped in sterile water, were planted in the open. Both lots flowered fairly well in the following spring, and all the bulbs appeared to be sound when lifted. Two of the inoculated bulbs rotted with *Fusarium* during storage in the following summer, but all the control bulbs remained sound. It is possible that infection had persisted in the soil through the growing period, but the number rotting was too small to be significant.

From the data given in Tables I, II and III, it is concluded that *F. bulbigenum* is able to attack living roots of *Narcissus* bulbs in the autumn under suitable conditions, *i.e.* when the roots are kept moist at a temperature of 20–30°C. Moreover, under the same suitable conditions,

the fungus can completely destroy the roots and penetrate the bulb by way of the dying roots.

The failure of the fungus to parasitise the roots when the bulbs were planted in the open may probably be attributed to the low temperature of the soil. McWhorter and Weiss⁽³⁾ state that, under American conditions, *Fusarium bulbigenum* attacks the bulb in the soil, through the roots and basal plate, when the soil temperature rises above 70°F. Soil temperatures at three typical bulb-growing areas in England during 1931 are available⁽⁴⁾, and in no case did these temperatures rise to 70°F. during the growing season. On this account it is probable that invasion of the bulbs in the soil through the growing roots does not normally occur under English conditions.

So far the invasion of the bulb through the young roots has been considered. It is possible that, in an unusually hot summer, the fungus might be able to penetrate the bulb through the dying roots at the end of the growing season, *i.e.* in May or June. Accordingly fifty bulbs of variety Evangeline were lifted on May 18th, 1934, and were heeled into the soil to allow any damaged roots to recover. On May 31st the bulbs were again carefully lifted. The roots of twenty-five of these were dipped in a spore suspension of *F. bulbigenum*, those of the remaining twenty-five being dipped in sterile water. The bulbs were then heeled into the soil again until July 16th, when they were lifted and stored at laboratory temperature. On the same day fifty bulbs of the same stock, which had not been previously disturbed, were lifted and the roots of twenty-five were dipped in spore suspension and those of the remaining twenty-five dipped in sterile water. After storage for 2 months, six bulbs of the lot treated with spore suspension on May 31st and one of those treated with sterile water on the same date had rotted with *Fusarium*. The remainder were sound.

This experiment, though by no means conclusive, suggests that infection does take place through the roots at the end of the growing period in an unusually hot season, such as occurred in 1934. It is probable that a higher proportion of infection would have been obtained if the soil had not been very dry. The failure of infection of the bulbs treated on July 16th may have been due to unsuitable conditions for spore germination, since no attempt was made to keep the roots moist. It is hoped to carry out more experiments along these lines, using more susceptible varieties.

(ii) *Infection of bulbs during hot-water treatment.*

Reference has been made to two experiments by Gregory in which bulbs were attacked after being dipped in a suspension of *Fusarium* spores and stored at a fairly high temperature. In one of these experiments bulbs of variety Golden Spur were immersed in water with *Fusarium* spores at 36°C. for 4 hours. In the other, bulbs of variety Henry Irving had their bases dipped in a cold suspension of *Fusarium* spores and were stored in a moist condition at 27°C. Though Gregory does not state exactly at what time of year these experiments were carried out, it is clear that it must have been in the late autumn, as he noted the prominence of the root initials. (The bulbs must have passed through their phase of maximum dormancy.) The writer's investigations were therefore directed in the first instance to a study of the effects of the hot-water bath on bulbs at this time of year.

The possibility that infection could take place from spores present in warm water appeared to be of the greatest practical importance, since hot-water treatment, in which bulbs are bathed for 3 hours in water at a temperature of 42–43°C., is a routine measure carried out by growers in order to free the stocks from the bulb eelworm, *Anguillulina* (*Tylenchus*) *dipsaci*.

The general method of experiment was as follows. Bulbs were bathed for the standard time at 42°C. with or without the addition of *Fusarium* spores to the water. Untreated bulbs provided a second type of control. The bathed bulbs were stored at 30°C. under continuous moist conditions in closed dishes, or in open dishes so that they dried off superficially within a few days of treatment.

In a typical experiment, batches of ten bulbs of the variety Spring Glory were treated as above described, with the results shown in Table IV.

Table IV.

Treatment	Storage conditions	No. of bulbs attacked
Hot water treated with <i>Fusarium</i> spores	30°C. moist	10
" " without <i>Fusarium</i> spores	30°C. "dry"	10
" " "	30°C. moist	4
" " "	30°C. "dry"	1
No treatment	30°C. dry	0

It is seen that contamination of the bath with *Fusarium* spores led to destruction of all the bulbs whether the storage conditions were moist or "dry". Presumably in the case of "dry" storage the loss of moisture from the bathed bulbs was sufficiently slow to allow attack to become established, so that the fungus became independent of the superficial

drying of the bulbs. The loss in the case where no *Fusarium* spores were added to the bath means either that spores became distributed in the bath from naturally contaminated bulbs or that latent infection was reawakened by the uptake of moisture. Thus some contamination or infection was present in this stock, and the fact that the untreated controls were not attacked, even at the relatively high temperature of storage, must be attributed to the thoroughly dry condition of the unbathed bulbs.

The collected results of experiments in this connection are shown in Table V, in which the last column gives the numbers of bulbs attacked within 14 days. It is clear that practically all the bulbs succumb to hot-water treatment with the addition of spores to the water, whereas the controls show very little loss.

Table V.

Variety	Treatment	No. of bulbs used	No. of bulbs attacked
Spring Glory	Hot water treated with spores	200	191
"	No treatment	200	3
Victoria	Hot water treated with spores	100	96
"	No treatment	100	8
Emperor	Hot water treated with spores	10	9
"	No treatment	10	0
Grandis	Hot water treated with spores	30	29
"	No treatment	30	7
Poeticus Ornatus	Hot water treated with spores	30	29
"	No treatment	30	2
Seagull	Hot water treated with spores	30	15
"	No treatment	30	0
Golden Spur	Hot water treated with spores	10	8
"	No treatment	10	0
Henry Irving	Hot water treated with spores	10	10
"	No treatment	10	0
Total	Hot water treated with spores	420	387
"	No treatment	420	20

In one experiment, as mentioned above, Gregory records a high proportion of attack in bulbs which had had their bases dipped in a suspension of *Fusarium* spores, presumably at laboratory temperature, and which were subsequently stored at 27°C.

Experiments were carried out using the varieties Emperor, Spring Glory and Glory of Leiden, and it was found that soaking the bulbs in a cold spore suspension or merely dipping them in the suspension led to a high proportion of attack when they were incubated at 20–30°C. If the bulbs were soaked in hot water before being dipped in the cold spore suspension the amount of attack was increased, probably through the softening of the tissues of the basal plates.

A complete record was kept of the position and extent of the infected area in the majority of the bulbs used in the hot-water experiments (collected in Tables IV and V). The bulbs were all examined after 14 days' storage (with the exception of those in one experiment, where 21 days elapsed between treatment and examination). This period, although sufficient to allow infection to become established, was short enough to ensure that the point of origin of attack was still determinable in the majority of cases. Most of the infected bulbs showed rotting progressing from the base upwards. In a few the disease was spreading from the "nose" downwards, while others were too badly rotted for the point of entry of the fungus to be determined. Exact figures are given in Table VI.

Table VI.

Variety	Total of bulbs	Rotted from base	Rotted from nose	Rotted from wound	Damaged by fly	Badly rotted
Spring Glory	224	144	4	0	.8	68
Victoria	94	45	1	2	0	46
Golden Spur	8	5	1	0	0	2
Henry Irving	10	9	0	0	0	1
Total	336	203	6	2	8	117

The data given in Table VI provide evidence in support of Gregory's hypothesis that infection resulting from hot-water treatment takes place *via* the natural wounds made by the emergence of the young roots. Confirmatory evidence was obtained from a rather different type of experiment carried out with offsets of Spring Glory, Golden Spur, Henry Irving and Victoria. These bulbs were treated with a mixture of paraffin wax and vaseline in the following ways: (i) whole bulb dipped in wax, (ii) basal plate covered with wax, (iii) basal ring covered with wax, (iv) nose covered with cellophane which was sealed down with wax. Others were left untreated. These bulbs were then subjected to infection, either through hot-water treatment in the presence of spores or by dipping in a cold spore suspension. The results obtained after incubation at 30°C. for 14 days showed a considerable decrease in the number of bulbs attacked when the basal plates or basal rings were protected by wax. In other cases different parts of the offsets were "painted" with a thick suspension of *Fusarium* spores, and it was found that the percentage of attack was greatest when the basal plates were so painted.

The fact that recent breaking apart of the components of double-nosed bulbs may be responsible for increased losses resulting from the hot-water treatment, was established by experiments with Spring Glory and Victoria.

The data given in Tables IV, V and VI refer to experiments carried out in the late autumn when the bulbs are at the end of their dormancy period and the roots are beginning to emerge naturally. Having regard to Gregory's suggestion that penetration may take place at the point of exit of the young roots, it was desirable to extend the work over a larger portion of the normal storage period. A series of tests was therefore carried out from July onwards during two summers.

Batches of twenty-five bulbs of the susceptible variety Spring Glory and of a more resistant double *Incomparabilis* variety were hot-water treated, with the addition of *Fusarium* spores to the water, at intervals of about 3 weeks from mid-July to early October. The treated bulbs, together with the same number of untreated controls, were stored in open trays, some at 27°C. and others in an unheated room. Table VII gives the number of bulbs (out of twenty-five) which rotted within 6 weeks from the date of treatment. The control bulbs in all cases remained sound.

Table VII.

Variety	Storage conditions	Date of treatment				
		July 17	Aug. 8	Aug. 29	Sept. 20	Oct. 6
Spring Glory	27°C.	15	12	4	18	23
"	Room temp.	9	3	0	5	1
Incomparabilis	27°C.	2	0	0	3	—

The room temperatures prevailing during the period of the various experiments were determined by a maximum and minimum thermometer and were as follows:

During experiment of July 17th	13–30°C.
"	August 8th	...	11–27°C.
"	August 29th	...	9–26°C.
"	September 20th	...	4–22°C.
"	October 6th	...	2–19°C.

The results obtained with Spring Glory bulbs when storage was at 27°C. indicate a clear minimum of susceptibility in the batch treated on August 29th.¹ The same conclusion is also suggested by the behaviour of the bulbs stored at room temperature. Throughout the period of the experiments the room temperature was falling, and this in itself would help to explain the drop in numbers of attacked bulbs from July 17th to August 29th, although the temperature during the period of the experiment of August 29th still reached a level suitable for the rotting of susceptible bulbs. The increase in the number of attacked bulbs in the

¹ This result has been confirmed by experiments carried out during the summer of 1935.

lot treated on September 29th occurred in spite of a further fall in temperature.

The behaviour of the *Incomparabilis* variety is in general agreement with that of the variety *Spring Glory*. The numbers of rotted bulbs are much smaller, in accordance with the greater resistance of this variety. These results are shown graphically in Fig. 1.

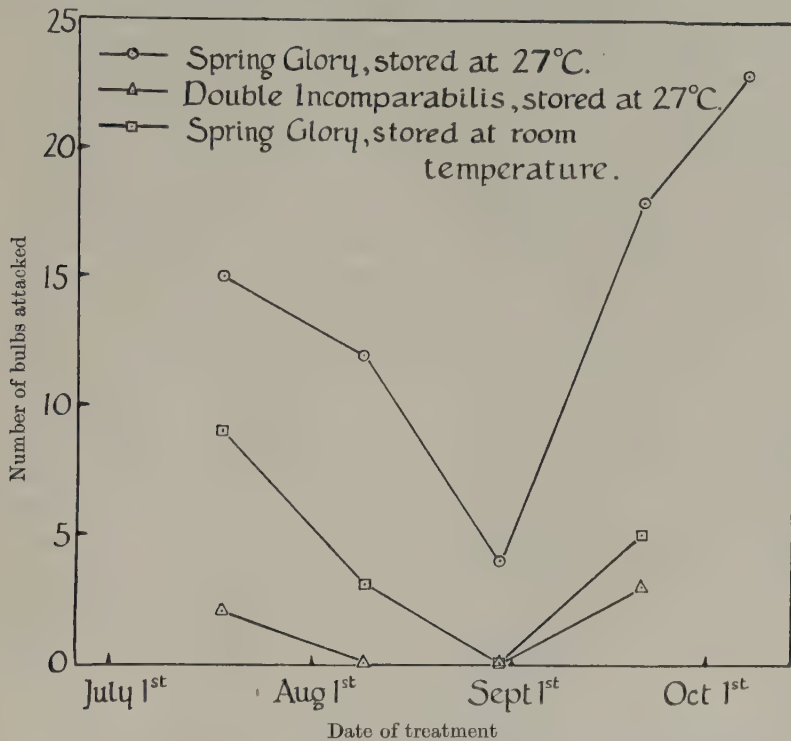


Fig. 1. Graph showing the changes in susceptibility of bulbs to spores present in the hot-water bath during the normal period of storage.

Similar experiments during the previous summer with the variety *Spring Glory* in which the bulbs were stored at room temperature gave comparable results. The number of bulbs (out of twenty-five) rotting after treatment being as follows:

Treated July 28th,	room temperature 14–31°C.,	20 bulbs rotted
„ August 28th,	„ 10–30°C.,	2 „
„ September 29th,	„ 5–20°C.,	1 „
„ October 27th,	„ 1–17°C.,	3 „
„ November 27th,	„ 0–17°C.,	7 „

The relationships indicated are essentially the same as those of Table VII, *i.e.* a fall in the number of bulbs attacked during the early part of the storage season followed by a rise in the later phases, and that in spite of continuously falling temperature.

Similar tests carried out with the susceptible variety Glory of Leiden and the rather more resistant Barri Conspicuus and a double Incomparabilis variety, while in general agreement with the above, were not carried out in sufficient numbers to warrant a detailed description.

The stocks used showed only a negligible loss from *Fusarium* rot when stored in open trays in the unheated shed, during the period of the experiments. Hence the initial fall in the number of bulbs rotting after treatment cannot be ascribed to the removal of infected bulbs from the stocks.

Thus susceptibility of the bulbs to infection from spores present in the hot-water bath is high during the early part of the storage period (*i.e.* during July and the first week in August) but is much less during the latter part of August and in September. It increases again during the early autumn until approximately 100 per cent. of bulbs, hot-water treated in the presence of *Fusarium* spores, are attacked (under suitable temperature conditions) if treatment takes place in October or later. This period of minimum susceptibility probably corresponds with that of maximum dormancy (described by Gould (1)), and is probably similarly dependent on such factors as season, locality and variety of bulb used. During the earlier period of high susceptibility the fungus may enter the bulb either by way of the old roots or the scars where these have been removed (either naturally or in the process of handling the bulbs) or by way of the nose of the bulb where dead foliage has been recently removed. The former is more likely to be the case, since rotting was usually found to progress from the base. The old roots had died and their scars were apparently quite healed by the end of August, while the new roots were not visible. By the end of September these showed as projections round the basal ring of the bulbs and by October some of them had broken through. It was thus probable that the fungus had entered bulbs treated in September or later by way of the natural wounds made by the emergence of the roots or by way of the roots themselves. In no case was the rate of emergence of roots altered by hot-water treatment. The date of root emergence varied with the variety of bulb used, those of the early flowering variety Spring Glory being somewhat in advance of those in the other three later varieties.

A distinct correlation can thus be traced between the degree of dormancy of the bulbs and their susceptibility to attack resulting from

hot-water treatment. Since this operation is usually carried out during the period of maximum dormancy, the risk of loss through bathing is less than would be expected.

An experiment was performed in October to test the effects of quick drying after hot-water treatment. Offsets of variety Spring Glory were hot-water treated in the presence of *Fusarium* spores and were then dried in the following ways: (a) spread on wire netting with free passage of air under the netting for 24 hours, (b) spread on dry newspaper on the laboratory bench for 24 hours, (c) heaped on the bench. The bulbs were then stored at 27°C. and were examined after 6 weeks, when the numbers found to be rotted in each case were (a) 14 (out of 25), (b) 15 and (c) 21.

Quick drying and cooling after hot-water treatment are recommended by McWhorter and Weiss⁽³⁾ in order to avoid "heating up" of the wet bulbs and subsequent heavy losses from *Fusarium* rot. In England it is said to be the usual practice^(4, 5) to leave the bulbs in the bags for 12-24 hours, after removal from the hot-water bath, in order to cool them slowly, in the belief that rapid cooling is detrimental. The writer has been unable to obtain any evidence that this belief is founded on fact. Moreover, in experiments, to be described later, in which large numbers of bulbs were hot-water treated and stored for a time before being planted, no case of injury from quick drying occurred. In the light of the results described above it is obvious that the practice of leaving the bulbs in the bags provides ideal conditions for attack by *Fusarium*. Although more rapid drying is, therefore, to be recommended, it does not afford a control of the disease, since it is not possible to dry the bulbs quickly enough entirely to prevent attack.

III. CONTROL MEASURES.

Gregory (*loc. cit.*) suggested three possible types of control of the *Fusarium* disease, namely fungicidal treatment, attention to conditions of storage and early planting, and carried out a preliminary investigation of all three methods. Attention has since been chiefly concentrated on fungicidal treatment, and the results obtained are described below. Experiments are also in progress along the two other lines indicated, but the work is not sufficiently advanced for publication.

Treatment with fungicides.

The attempt to control the *Fusarium* disease by the inclusion of fungicides in the hot-water bath is naturally suggested by the desirability of combining two operations in one. The hot-water treatment, however,

can only be carried out with safety at about the period of maximum dormancy of the bulbs. As will appear later, this is a severe limitation from the point of view of the control of the disease, since a good deal of damage may have occurred before the time when the hot-water bath may be safely used.

Apart, however, from such losses, there is, as has been shown above, the considerable risk of the bulbs being infected by spores of the fungus present in the bath, especially if the treated bulbs be subsequently stored at a temperature of 20°C. or higher. It is, of course, improbable that contamination of the bath with spores would, under practical conditions, be as severe as was arranged in the experiments described, but it is to be remembered that the losses obtained experimentally often approximated to 100 per cent. The risk of contamination in the way indicated is thus a very real one. On the basis of the conclusion reached above, that the bulbs pass through a phase of minimum susceptibility, the risk of infection is diminished if the bathing be carried out at the proper time. In practice this may not always be possible when a miscellaneous lot of bulbs has to be treated. Moreover, the risk of infection through wounds is probably as great during the period of dormancy as at any other time.

The hot-water treatment has a further disadvantage from the point of view of fungal attack. Soaking the bulbs at a temperature of 42°C. for 3 hours results in a considerable uptake of water with consequent reawakening of mycelium which was otherwise arrested. Abundant evidence that such an effect does occur has been met with in the course of this work. It was of interest, therefore, to determine whether this second source of infection could be checked by the incorporation of a suitable fungicide in the bath.

Gregory (*loc. cit.*) describes an experiment in this connection. Batches of Victoria bulbs were bathed in mid-September, with or without the addition of fungicides, and another batch was left untreated as a control. The bulbs were then stored for about 2 months, when it was found that the highest percentage of infection occurred in the batch which had been bathed in water alone. The addition of formalin (1 per cent.) or of "Uspulun" (0.25–0.5 per cent.) reduced the percentage of wastage to about half, which was approximately the same figure as was shown by the untreated control batch. The addition of the fungicide did not eliminate disease, but the result was to neutralise the harmful effect of the hot-water bath treatment so that the latter with fungicide gave much the same amount of wastage as no treatment at all.

McWhorter and Weiss (*loc. cit.*), working in America, report very

favourably on the efficiency of certain fungicides, when added to the hot-water bath, in controlling *Fusarium* (and other) diseases. The claim is made in general terms that the vigour of the plant is improved, that the growing season is lengthened and that a marked increase, both in total yield and in the number of large bulbs, takes place. Unfortunately this paper gives no figures in support of these conclusions, and it is obvious from the text that favourable results are not always obtained from the treatment.

Experiments comparable to that quoted from Gregory's paper are summarised in Table VIII, the main difference being that, in order to give optimal conditions for the development of the disease, all the bulbs were stored for 14 days at 30°C. Half of the Victoria bulbs of each batch were stored in closed moist dishes so that they dried very slowly. The remainder and all the Spring Glory bulbs were allowed to dry off in open dishes. Since there was no material difference in the numbers which rotted under the two conditions, no distinction is made in the table.

Table VIII.

Variety	Treatment	No. of bulbs used	No. of bulbs attacked
Victoria	Hot-water treated	50	31
"	" + 0.1 % formalin*	50	18
"	No treatment	50	11
Spring Glory	Hot-water treated	25	19
"	" + spores	25	24
"	" + spores + 0.1 % formalin	25	0
"	No treatment	25	1

* The "formalin" used was 40 per cent. formaldehyde. Thus a 0.1 per cent. formalin solution would be equivalent to a 0.04 per cent. solution of formaldehyde.

Table VIII illustrates in varying degree the same type of results as that claimed by Gregory. With both varieties the addition of formalin to the bath definitely reduced the serious wastage arising from the bath treatment. In the case of Victoria the result was not quite so good as in the untreated controls; with Spring Glory it was as good. Further experiments with the four varieties, Spring Glory, Victoria, Grandis and Poeticus Ornatus, yielded results of the same type.

The data of Table VIII may be interpreted along the following lines. Since the percentage of formalin added is more than three times that required for the destruction of free-floating spores of the *Fusarium* (0.03 per cent. formalin for 2 hours in the bath is, according to Gregory, toxic to all the spores), no spread of contamination could take place in the treatments where formalin was present. One must assume, therefore, that in a batch of fifty Victoria bulbs there were eighteen in which the

infection was so deeply seated that the chemical was unable to reach the fungus in the time available. The Spring Glory bulbs, however, were either merely contaminated on the surface or the lesions were sufficiently shallow to allow of complete sterilisation. The difference between the number of bulbs attacked when bathed in water alone and when bathed with the addition of formalin to the water, *i.e.* 13 (31–18) for Victoria and 19 (19–0) for Spring Glory, give a measure, either of the numbers of bulbs which were superficially infected at the start, or which became contaminated in the course of the bathing treatment. These two effects cannot be distinguished, and it is probable that both play a part. Finally there were eleven (out of fifty) of the Victoria bulbs in which the infection had gone so far that the favourable temperature was sufficient to lead to rotting in the absence of any soaking in water. The corresponding number for Spring Glory was much less, *viz.* one out of twenty-five.

The failure of a fungicide to stop invasion which had visibly begun was further illustrated by tests in which bulbs in that condition were subjected to various hot or cold steeps in formalin of strength ranging from 0.5 to 2.5 per cent. In no case did the treatment retard the rate of advance of the fungus.

Under the somewhat drastic conditions of storage used in these experiments, it is thus perfectly clear that the addition of a fungicide to the hot-water bath is to be recommended, even though this does not, as a rule, lead to the elimination of the fungus.

During the period 1931–5 experiments similar to those of Table VIII, but with the modification that the treated bulbs were stored for some time at the temperature prevailing in an unheated room, were carried out on a considerable scale, at the Imperial College Field Station, Slough. For reasons which are considered below, these experiments furnished very little information regarding the efficacy of fungicides in controlling *Fusarium* disease. Substantial evidence has, however, been obtained on the effects of various treatments on growth and flowering and on increase in bulb weight. Beyond an indication of the methods and scope of these experiments, the results will be presented in summarised form.

During the four seasons in question, a stock of approximately 6000 bulbs was used in this connection. This consisted chiefly of the varieties Spring Glory and Victoria together with smaller lots of the varieties Golden Spur, Henry Irving, Emperor, Glory of Leiden, Grandis, Obval-laris, Barri Conspicuous, Seagull, Evangeline, Poeticus Ornatus, Poeticus Ornatus Maximus and a double Incomparabilis variety.

In each year about one-half of the stock was used for experimental pur-

poses. The treatments were applied in the months of July to September. The usual batch for any one treatment was fifty bulbs, but varied in different experiments from twenty-five to 150. A few plantings were made at once in the open, but the majority of the batches were stored at ordinary temperatures for some time before planting, the condition being then noted. In the following year observations were made of the number of bulbs missing, of the character of the foliage, of the amount and quality of the flowers and of the period of flowering. The bulbs were lifted at the normal time, dried, weighed and stored under ordinary conditions in shallow trays until planting time. Any losses were recorded and finally all the apparently sound bulbs of each variety were lumped together and planted in stock beds. Meanwhile the other half of the stock had been lifted and dried, and this constituted the material for the second year's experiments; and so on in rotation.

From time to time new stocks of bulbs were obtained. These were in general selected as being stocks with a bad history, but on closer acquaintance it was found that usually a number of troubles (root-plate rot, stripe, and rather frequently eelworm and fly damage) were present. In such cases the effects of the treatments used are best described as relating to general inferiority of stock and not to *Fusarium* trouble in particular. A few of the stocks were of good quality initially and others have been improved by removal of bulbs with defective basal plates, by rogueing out specimens with striped foliage during the growing season while the bulbs were in the stock beds, and by the various bathings which have been given from time to time.

The behaviour of a good stock of Spring Glory will be described as an illustration. The stock was suspected of *Fusarium* trouble and was obtained from Holland, the experiment being carried out as soon as the bulbs were received. The bulbs were divided into batches of fifty and treated on August 18th to 21st, three batches being used for each treatment. The treatments were as follows:

- (1) No treatment, planted August 19th.
- (2) No treatment, planted September 16th.
- (3) Hot-water treated, planted September 16th.
- (4) Hot-water treated with the addition of 0.5 per cent. formalin to the water, planted September 16th.
- (5) Five hours' cold steep (18.5°C.) in 0.5 per cent. formalin, planted September 16th.
- (6) Five hours' cold steep in 0.1 per cent. solution of mercuric chloride, planted September 16th.

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Very few of these bulbs, which were stored at room temperature, rotted between the dates of treatment and planting, but untreated bulbs of the same stock which were stored at 31°C. showed heavy losses and *Fusarium* was isolated from them. Thus, although the stock was apparently infected with the *Fusarium*, the experiment yielded no data on the effects of these treatments on the control of the disease in bulbs stored at ordinary temperatures. It must be concluded, therefore, that the temperature of the room was low enough to check the advance of the fungus.

Flowering in the following spring was good and there were only two bulbs missing out of the lot. The formalin treatment had no harmful effects on the plants, but the mercuric chloride treatment retarded the flowering by a few days.

The bulbs were lifted in the following July and, after preliminary drying and cleaning, were weighed. The increases in weight are given in Table IX, and it will be seen that the incorporation of formalin in the hot-water bath led to an increased gain in weight. The cold treatments had no such effect. Twenty-two experiments of this type were carried out during the four summers 1931-4. These will be described from the point of view of (a) effect on *Fusarium* disease, (b) effect on growth and flowering, and (c) effect on increase in weight of bulbs during the growing season.

Table IX.

Treatment	No. of bulbs used	Weight when planted lb.	Weight when lifted lb.	Gain in weight lb.
No treatment	150	16.95	27.25	10.3
Hot-water treated	150	16.65	26.25	9.59
„ + 0.5% formalin	150	16.48	30.75	14.28
Cold soak, 0.5% formalin	150	17.33	27.5	10.17
„ 0.1% mercuric chloride	150	17.25	27.5	10.25

(a) *Effect on Fusarium disease.*

Very little trouble of this nature was encountered. The number of bulbs which rotted in storage or in the ground was generally insignificant, so that no conclusion could be drawn regarding the value of any fungicidal treatment. Only in two experiments, with the varieties Glory of Leiden and Obvallaris, did any appreciable loss occur in bulbs which had been hot-water treated. The addition of formalin to the bath in these cases had a beneficial effect. Thus, in batches of 200 Glory of Leiden bulbs, the addition of 0.5 per cent. formalin to the hot-water bath in mid-September reduced the number of blanks in the following spring

from nineteen to ten. The number rotting in storage from *Fusarium* in batches of forty Obvallaris bulbs treated in late July was similarly reduced from six to none.

It is clear that some of the stocks used were originally contaminated with the *Fusarium*, since sample batches stored at 30°C. developed the disease. An explanation of the failure to rot of bulbs stored at room temperature is to be sought either in the storage conditions or in the soil conditions under which the bulbs were grown.

It was thought possible that the soil at Slough was either not heavily contaminated with the *Fusarium*, since the plots used had been recently cut out of old meadow land, or that the soil was unsuitable for the growth of the fungus. In order to test the latter hypothesis Victoria bulbs, from a stock which had shown considerable loss while stored in a commercial warehouse, were planted, some in the light sandy beds at Slough, and others in a heavier soil. Bulbs from a number of other suspected stocks were planted in a heavy loam or in a clay soil. All these were lifted in the following year, dried in the usual way and stored during the summer. There was no material loss in any case despite high average temperatures. Moreover, the fact that bulbs obtained from Holland and put into storage did not show any rotting, although heavy losses in sample batches stored at 30°C. showed that these were contaminated, suggested that storage conditions rather than soil conditions were responsible for the small amount of attack.

Several of the experiments under consideration were begun rather late in the storage period (September–October) at which time the temperature of the storage shed or of the ground was too low for development of the disease. The fact that bulbs treated in July of 1932 and in August of 1933 and 1934 remained sound cannot be explained in the same way. In these cases storage temperatures were high.

An alternative hypothesis was that the method of cleaning and drying was such that the chances of invasion were curtailed. The bulbs on harvesting were placed in shallow trays, and as the individual batches were not large (usually 50–100) there was rarely more than one layer of bulbs in each tray. The trays were stacked in a well-ventilated shed in such a manner that there was free passage of air between them. Since the Slough soil is particularly light, the bulbs were fairly clean when lifted and they dried rapidly. It is probable that this rapid drying prevented the fungus from invading the tissues of superficially infected bulbs.

This view is favoured by a number of tests in which bulbs which had dried off and which had shown no wastage over the storage period were

proved to harbour the fungus by the simple expedient of soaking them in water and storing at 20–30°C. Under these conditions a significant number became rotted with *Fusarium*, while untreated control batches remained sound. For example, out of batches of twenty-five Spring Glory bulbs, from a stock which had remained sound during summer storage, nineteen rotted with *Fusarium* after hot-water treatment, ten after an hour's soaking in cold water, and only one of the untreated lot when stored at 30°C.

(b) *Effects on growth and flowering.*

Notes were taken each year of the appearance and state of advancement of the foliage and of the date at which it died down. During the

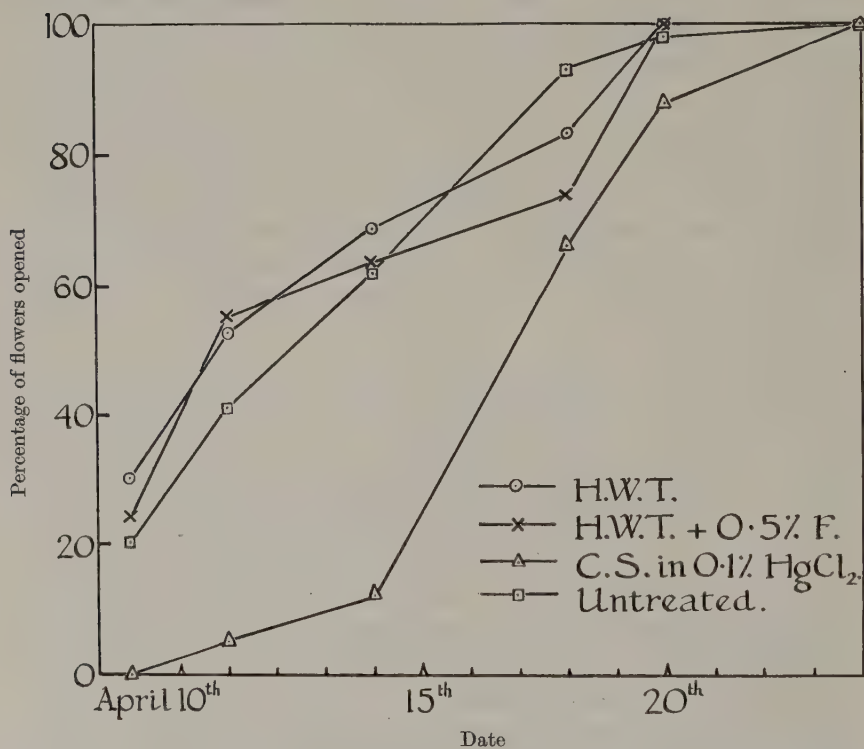


Fig. 2. A typical flowering chart, showing the effects of various bulb treatments on the rate of flowering in the following spring. H.W.T. = hot-water treated, F. = formalin, C.S. = cold soak.

flowering season the open flowers (*i.e.* with perianth fully expanded) were cut at frequent intervals and the number noted. The numbers of

blind buds and of deformed or dwarfed blooms were also recorded. Complete records are available over the years 1932-5.

In no case did the inclusion of 0.5 per cent. formalin in the hot-water bath cause any harmful effect on the foliage of any of the varieties used. During the season 1932-3 the dose was increased in some experiments to 1.5 per cent., again with no resulting damage. The date at which the foliage died down was, so far as could be seen, unaffected by any of the treatments given.

The date of flowering was in general unaffected by the formalin treatments, although in a few cases there was a slight advancement. The application of a mercuric chloride cold steep (0.1 per cent. for 5 hours) had a retarding effect on emergence and growth of foliage and on date of flowering. As there was no obvious countervailing advantage, the mercuric cold steep was dropped after the first year's experiments. None of the treatments affected the number or quality of flowers produced.¹

A typical flowering chart is shown in Fig. 2, in which percentage number of flowers is plotted against date of flowering for a typical experiment with the variety Spring Glory. The batch treated with a mercuric chloride cold steep shows a distinct retardation of flowering when compared with the untreated controls. The rate of flowering of the hot-water treated lots, with or without formalin, is approximately the same as that of the controls.

(c) *Effect on increase in weight of bulbs during the growing season.*

An illustration of the actual increase of bulb weight obtained when various treatments were used has already been given in Table IX. The results of all the experiments in this connection are summarised in Table X. The treatments given are compared in pairs as indicated in the first column. Thus in the first line of the table the hot-water treatment with 0.5 per cent. formalin added was compared with no treatment in twelve experiments. Four of these gave a large increase (in the amount of gain in weight during the growing season) in favour of the former treatment, seven a slight increase and one a slight decrease; and so on. A large increase or a large decrease was arbitrarily taken as one in which the difference was at least 1 lb. per 100 bulbs. A light increase or decrease was one of less than this amount.

The data given in Table X must be interpreted with caution, since in a few of the stocks used a certain amount of eelworm and fly trouble

¹ In two cases splitting of the flowers resulted from hot-water treatment carried out in late July, but those bathed with formalin were no worse than the water controls.

Table X.

Treatments compared	No. of trials	Increase		Decrease	
		Large	Slight	Slight	Large
H.W.T. + 0.5% F. <i>v.</i> no treatment	12	4	7	1	0
H.W.T. + 0.5% F. <i>v.</i> H.W.T.	16	3	8	3	2
H.W.T. + 0.5% F. <i>v.</i> H.W.T. + 1.5% F.	6	1	4	0	1
H.W.T. + 0.5% F. <i>v.</i> C.S. in 0.1% HgCl ₂	10	6	2	2	0
H.W.T. + 1.5% F. <i>v.</i> no treatment	6	2	3	1	0
H.W.T. + 1.5% F. <i>v.</i> H.W.T.	6	2	2	1	1
H.W.T. <i>v.</i> no treatment	12	4	5	3	0
C.S. in 0.1% HgCl ₂ <i>v.</i> no treatment	10	4	4	2	0
C.S. in 0.5% F. <i>v.</i> no treatment	1	0	0	1	0

H.W.T. = hot-water treatment; F. = formalin; C.S. = cold soak.

was present. Thus the good effects of hot-water treatment and of this with the addition of formalin may be in part attributed to the effect of the treatments in controlling these pests. The superiority of bathing with formalin to bathing in water alone cannot be explained in this way. It is doubtful, however, whether this superiority is due to the effect on basal plate trouble (which was originally prevalent in most of the stocks used), or whether the formalin treatment is in some way beneficial to healthy bulbs. It is clear, nevertheless, that the addition of formalin to the hot-water bath not only does not decrease the normal gain in weight of bulbs, but in many cases causes a substantial increase, and is to be preferred to bathing in water alone. Hot-water treatment with 0.5 per cent. formalin was usually more satisfactory than similar treatment with 1.5 per cent. formalin. Cold steeps in 0.1 per cent. mercuric chloride were rather better than no treatment, but were definitely less effective than hot formalin treatment. Since only one trial of the effect of a cold steep in formalin was included in these experiments no conclusions can be drawn as to the effect of this treatment.

The main conclusion to be drawn from these experiments is that formalin may be added to the hot-water bath, up to a concentration of at least 1.5 per cent., without any harmful effects on growth and flowering of the bulbs. Further experiments along the same lines are projected, and it is hoped to obtain more suitable stocks for this purpose so that more satisfactory data on the question of the effects on the incidence and spread of the *Fusarium* disease may be obtained. It is improbable, however, that a complete control of the disease can be achieved by the incorporation of fungicides in the hot-water bath, since, in order to avoid damage to foliage and flowers, this treatment must be carried out rather late in the storage season, *i.e.* in late August. Some evidence has been obtained, from a study of bulbs under commercial conditions, that a

considerable amount of loss from *Fusarium* takes place before this date. It is likely that attack begins soon after lifting, while the bulbs are still damp and conditions are thus suitable for the growth of the fungus. Attack is thus sufficiently advanced in an infected stock by the time the bulbs are hot-water treated (even though they may not be visibly rotted) that fungicidal treatment at this time is too late to save a large number of them. Thus while the incorporation of a fungicide in the bath is desirable to prevent the further *spread* of the disease, yet fungicidal treatment would probably be best applied within a week of lifting. For reasons already cited it is not practicable to carry out hot-water treatment so soon after lifting, and any fungicidal treatment would have to take the form of cold steeping or dry dusting. McWhorter and Weiss (*loc. cit.*) claim good results from certain treatments of this type, but again do not give any figures in support of this claim. Such cold steepings as are described in the present paper took place rather late, *i.e.* late July to mid-September, and the evidence obtained is thus of little importance in this connection. A series of experiments on a scale similar to that of the hot-water treatment tests described above is in progress but complete results are not yet available.

IV. SUMMARY.

1. Experiments on the inoculation of roots of *Narcissus* with *Fusarium bulbigenum* in the autumn show that, under suitable conditions of moisture and rather high temperature, the fungus is able to penetrate and destroy the roots of all varieties tested. Under similar suitable conditions it can penetrate bulbs of susceptible varieties *via* the parasitised roots. It is concluded that temperature conditions in England are seldom favourable to such penetration *via* the young roots during the autumn.

2. Some evidence is given that penetration of the bulbs may take place *via* the old roots at the end of the growing season when soil temperature is likely to be more favourable to attack. Further work on this point is desirable.

3. Gregory's statements that heavy losses from *Fusarium* may follow the standard hot-water treatment against bulb eelworm, when carried out in the autumn, and that in such cases penetration usually takes place at the base of the bulb are confirmed and amplified.

4. Experiments in which bulbs were hot-water treated, with the addition of *Fusarium* spores to the water, at intervals during the storage

period, indicate that bulbs pass through a phase of minimum susceptibility in late August and early September, *i.e.* at the normal time of bathing.

5. It has been shown that, where bulbs are hot-water treated in the presence of *Fusarium* spores, the addition of formalin (0.1 per cent. or more) to the water materially reduces the resulting losses.

6. A comprehensive series of experiments, with fourteen varieties, has been carried out during four seasons in order to test the effects of the addition of fungicide to the hot-water bath. These experiments, for reasons which are discussed fully in the text, have not yielded any conclusive evidence on the effect of this treatment on the spread of the disease. It has been amply shown, however, that the incorporation of 0.1–1.5 per cent. formalin in the bath has no harmful effects on foliage, date of flowering, quality and number of blooms or amount of increase in weight of bulbs during the growing season, but is often beneficial.

7. Cold steeps in 0.1 per cent. mercuric chloride solution led to a retardation of the date of flowering.

8. It is desirable that fungicidal treatment should take place before the normal date of hot-water treatment, and experiments are in progress to test the efficacy of various cold steeps and dry dustings carried out in the early part of the storage season.

In conclusion the writer wishes to express her thanks to Prof. W. Brown, who suggested this work, for much advice and assistance during its progress. Thanks are also due to Dr A. K. Ghamrawi who carried out some of the observations in the 1931 experiments.

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STUDIES ON EUROPEAN FOUL BROOD OF BEES

I. A DESCRIPTION OF STRAINS OF *BACILLUS ALVEI*
OBTAINED FROM DIFFERENT SOURCES, AND OF
ANOTHER SPECIES OCCURRING IN LARVAE
AFFECTED WITH THIS DISEASE

BY H. L. A. TARR.

*(From Rothamsted Experimental Station and the Lister Institute
of Preventive Medicine, London.)*

(With Plate XXVIII.)

BAUMGARTNER (14), working in Morgenthaler's laboratory on European foul brood, was able to isolate four distinct strains of bacteria, similar to *Bacillus alvei*, from diseased larvae obtained from a number of different cases of this disease. In the United States Burnside (2, 6) discovered a type of brood disease which he termed "Parafoulbrood", and which is apparently caused by an organism closely related to, but culturally distinguishable from, *B. alvei*, namely *B. para-alvei*. So far nothing has been published with reference to the cultural, morphological or biochemical characteristics of these organisms. During the summer of 1934 the author isolated, in addition to an organism which appeared to be similar to *B. alvei* (4, 7, 20, 12, 13) in its morphology and gross cultural characteristics, two bacilli from different cases of European foul brood which grew in apparently pure culture from the remains of the dead larvae. Both these bacilli were culturally, morphologically and biochemically distinguishable from *B. alvei*.

In view of these facts it seemed of interest to examine cultures of *B. alvei* from different sources with a view to determining what variations might exist among them. Through the kind co-operation of other workers in this field cultures of *B. alvei* have been obtained from several different countries, and these have been studied together with four strains isolated by the author. The source of each culture employed is given on p. 710.

Before the experimental work was commenced all these strains were purified by plating on meat digest agar and picking the isolated colonies which developed. It was found necessary to "dry" the plates for about 4 hours at 37°C. in order to hinder the spreading of surface colonies which is extremely marked with all these organisms. In each case stock cultures were obtained by culturing the organisms for 7 days at 37°C. on egg agar and sealing the resulting spore-containing cultures.

Culture No.	Source
1	Isolated from the dried larval scales from a case of European foul brood from Perth, Australia; the material supplied by Miss Betts. The organism grew in apparently pure culture from the scales.
2	Isolated from the ropy remains of a larva from an English case of European foul brood. The organism grew from the diseased material in apparently pure culture.
3	Isolated in the same manner as culture 2 from another English sample of European foul brood.
4	Isolated from a dead larva from an experimental nucleus in which infection had been initiated by the insertion of the diseased comb from which the larva used in isolating culture 3 had been obtained.
5	A strain of <i>B. alvei</i> supplied by Dr Borchert, Germany.
*6	" " Dr Morgenthaler, Switzerland.
*7	" " " "
8	" " Dr Burnside, U.S.A.
9	" " " "
10	" " Dr Lochhead, Canada
11	" " " "

* These two strains were received with a note to the effect that they were not identical. They had been isolated from Swiss samples of European foul brood obtained from different sources.

The tryptic digest meat broth employed was prepared in general by the method of Douglas (5), and from this the agar (2 per cent.) and gelatin (10 per cent.) media were prepared. The pH of the agar and broth media was approximately 7.4, and of the gelatin 7.2. Nitrate broth was prepared by adding 0.1 per cent. pure potassium nitrate to the digest broth. Litmus milk and blood serum were prepared in the usual manner. A medium consisting of 2 per cent. Witte peptone, 0.5 per cent. sodium chloride and 0.1 per cent. litmus to which 1 per cent. of polysaccharide, sugar, alcohol or glucoside was added, was tubed and sterilised by steaming on each of three successive days (pH 7.0). This type of medium was employed in the study of acid production by the various strains. The "egg agar" (egg-yeast-carrot-peptone agar) was prepared by the method of Sturtevant (17), the reaction being pH 7.0. Hartley's meat digest broth (8), prepared more concentrated than usual (approximately 198 mg. amino nitrogen per 100 ml. of finished broth) by increasing the proportion of meat hydrolysed, was used in detecting the production of indole. This broth, with the addition of 0.1 per cent. cystine, was used to study hydrogen sulphide formation. All these media were tubed in approximately 5 ml. amounts, and, with the exception of carbohydrate media, milk and serum, were sterilised by autoclaving.

A relatively uniform method of inoculation was employed. A trace of growth from an old spore-containing egg agar culture of the bacillus

in question was inoculated into a tube of broth which was incubated for 12 hours at 37°C. Liquid media were inoculated with 0.05 ml. of such a broth culture. Agar and gelatin slopes, serum, and gelatin stabs were inoculated directly from the broth cultures with a straight platinum needle. Nitrate reduction was studied after incubation of the cultures from 12 hours up to 1 week at 37°C., employing the Griess-Ilsovoy reagent to detect nitrite. Attempts to detect hydrogen sulphide were made by adding a few drops of a freshly prepared lead acetate solution to cystine broth cultures of the various bacilli after incubating them from 1 to 7 days at 37°C. Peptone water broth cultures containing sugars, etc., were incubated at 37°C. for 21 days, periodical examinations being made to ascertain whether or not acid was formed. Gelatin cultures were incubated at 21°C. Indole was tested for by shaking the culture with a small amount of ether, and then adding a few drops of Ehrlich's reagent.

The results obtained from the study of the eleven strains indicated that they could be divided into two groups as follows:

Group A. Cultures 1 and 2. These cultures were practically identical, but differed morphologically, culturally and biochemically from those of group B.

Group B. Cultures 3-11. These cultures could all be regarded as *B. alvei* as far as their gross cultural characteristics and morphology were concerned, but differed from one another on the basis of their fermentation reactions. The morphological, cultural and biochemical characteristics of these two groups will now be discussed.

GROUP A.

On agar the vegetative cells are very variable in size, long rods often occurring. Rods of 3-6 μ in length are most common. The average breadth is about 1.2-1.5 μ (Plate XXVIII, fig. 1). The ends of the vegetative cells exhibit a distinct tendency to be pointed, especially at the time of division. The organisms are actively motile by means of peritrichous flagella. Very young cells are Gram-positive, but the power to retain the stain is rapidly lost, and in older cultures all cells are Gram-negative. The method of sporulation is rather characteristic, a large oval cyst with one end rather pointed being formed (Plate XXVIII, figs. 2 and 3). There seems to be no tendency for the spores to arrange themselves in rows as in the case of strains of *B. alvei*.

Cultural characteristics.

In broth a uniform cloudy growth results and this does not increase appreciably after 24 hours. A light precipitate slowly settles but no ring or pellicle is formed. On agar slopes, dried for 1 week at 37°C. prior to inoculation with the hope of avoiding spreading, a soft, moist non-adherent growth results. In spite of drying the medium this spreads rapidly over the entire surface as a thin slightly opaque layer. In gelatin stab cultures a slow infundibularform liquefaction occurs, and in gelatin slope cultures a straight channel forms along the line of inoculation, the gelatin becoming completely liquefied in about 3 weeks. Litmus milk is slowly clotted with reduction of the litmus but with little or no formation of acid. The casein is slowly peptonised, the hydrolysis being almost complete in about 3 weeks. Blood serum is almost completely peptonised in 3 weeks. Rapid, spreading and abundant growth occurs on egg agar. In none of the above media is any appreciable odour produced.

Biochemical characteristics.

Both strains rapidly reduce nitrate to nitrite, but neither forms hydrogen sulphide. Apparently indole is not formed in broth or in 3-week-old milk cultures. The fermentation reactions of both strains are identical (Table I), though culture 1 ferments fructose very rapidly while culture 2 under identical conditions forms no acid in fructose during the first 10 days' incubation.

GROUP B.

The vegetative cells formed by the strains of this group all exhibit the same variability in size noted in the group A cultures, and also the same reaction toward Gram's stain. The cells are approximately the same average size as those of group A (Plate XXVIII, fig. 4). The manner of sporulation is, however, entirely different from that of cultures of group A. Spores are formed eccentrically, and while the rod may swell slightly at the site of spore formation, frequently no swelling occurs. The vegetative cells quickly disintegrate leaving free spores which are frequently arranged in long chains (Plate XXVIII, figs. 5 and 6). The young vegetative cells are sluggishly motile by means of peritrichous flagella (Plate XXVIII, fig. 7).

Cultural characteristics.

The growth in broth is rapid and a uniform cloudiness results. In ordinary broth no ring forms, but in Hartley's meat digest broth made

more concentrated than usual (*vide supra*) a definite ring is formed and a light fragile pellicle. On agar (dried) there is marked spreading, the growth appearing as a mass of colonies over the surface of the medium. The growth is soft, moist, and opalescent. In gelatin stab cultures the growth is typical, slow stratiform liquefaction occurs, and in the stab the organisms penetrate the gelatin causing a cloudy appearance, isolated colonies often being set up at some distance from the line of puncture. Characteristic growth also occurs on gelatin slope cultures. Surface growth first appears in the form of a large number of fine branched channels all over the gelatin surface, and the organisms penetrate below the surface of the medium causing a cloudy appearance. In about 2 weeks the gelatin is completely liquefied. In cultures 3, 4 and 9 the gelatin becomes yellow in colour, while in the other strains this appearance has not been noted. In litmus milk the indicator is rapidly reduced, but little or no acid is formed, the casein slowly clots and peptonisation occurs. Blood serum is slowly liquefied. Abundant growth results on egg agar. A very pronounced putrefactive odour, not unlike that of stale urine, is formed when strains of this group are cultivated on egg agar or in milk. This odour is not quite so pronounced when the bacilli are grown on meat agar or serum.

Biochemical characteristics.

Nitrate is not reduced to nitrite and hydrogen sulphide is not formed by any of the strains. Indole is formed by all the cultures when grown for long periods (2-3 weeks) in Hartley's broth or in milk, but not in ordinary broth. Definite growth of each of the strains investigated occurs in all the media which contain fermentable substances. There are, however, certain differences with reference to the fermentation reactions of the strains in group B (Table I), and it is on the basis of these variations that the question of the possibility of the multiplicity of species of *B. alvei* arises. All the strains ferment starch, dextrin, maltose, glucose, mannose, adonitol, glycerol and salicin, and all fail to ferment inulin, lactose, arabinose, xylose, mannitol, erythritol and dulcitol. There are, however, pronounced variations among the different strains with reference to their ability to ferment raffinose, sucrose, fructose, galactose and inositol. These variations are shown in Table II. It is of interest that the transfer of European foul brood to a fresh colony appears not to alter the fermentation reactions of the species of *B. alvei* present in the diseased larvae (cultures 3 and 4).

Table I.
The fermentation reactions of cultures of groups A and B.

Substrate	Number of culture and source										
	Group A		Group B								
	1 Australia	2 England	3 England	4 England	5 Germany	6 Switzer- land	7 Switzer- land	8 U.S.A.	9 U.S.A.	10 Canada	11 Canada
Starch	+	+	+	+	+	+	+	+	+	+	+
Dextrin	+	+	+	+	+	+	+	+	+	+	+
Inulin	-	-	-	-	-	-	-	-	-	-	-
Raffinose	-	-	+	+	+	+	-	-	-	+	+
Sucrose	+	+	+	+	+	-	-	-	-	+	+
Lactose	-	-	-	-	-	-	-	-	-	-	-
Maltose	+	+	+	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+	+
Fructose	+	+	-	-	+	-	+	+	+	-	-
Galactose	+	+	-	-	+	+	+	+	+	+	+
Mannose	+	+	+	+	+	+	+	+	+	+	+
Arabinose	-	-	-	-	-	-	-	-	-	-	-
Xylose	-	-	-	-	-	-	-	-	-	-	-
Mannitol	-	-	-	-	-	-	-	-	-	-	-
Erythritol	-	-	-	-	-	-	-	-	-	-	-
Inositol	-	-	+	+	-	+	+	+	+	+	+
Dulcitol	-	-	-	-	-	-	-	-	-	-	-
Adonitol	-	-	+	+	+	+	+	+	+	+	+
Glycerol	+	+	+	+	+	+	+	+	+	+	+
Salicin	+	+	+	+	+	+	+	+	+	+	+

+ = acid, - = no acid.

Table II.
The essential differences in the fermentation reactions
of the various strains studied.

Substrate	Group A Cultures 1 and 2	Group B				
		Cultures 3 and 4	Cultures 7, 8 and 9	Cultures 10 and 11	Culture 5	Culture 6
Raffinose	-	+	-	+	+	+
Sucrose	+	+	-	+	-	-
Fructose	+	-	+	-	+	-
Galactose	+	-	+	+	+	+
Inositol	-	+	+	+	-	+
Adonitol	-	+	+	+	+	+

+ = acid, - = no acid.

DISCUSSION.

It is, at present, impossible to classify with any accuracy the two cultures of group A. It is possible that the species is one hitherto undescribed; in any case it has not previously been described as occurring in larvae affected with European foul brood, and is quite distinct morphologically from *B. orpheus* (White (22)).

In their original work on *B. alvei* Cheshire and Cheyne⁽⁴⁾ gave an excellent description of the morphological and cultural characteristics of this organism, but made no reference to its fermentative powers. Harrison⁽⁷⁾ studied strains of *B. alvei* from nine different countries and stated that: "It is true that some of the cultures show certain differences, but they have not been sufficiently pronounced to even constitute a well-marked variety of the species." The fact that Harrison in this work stated that, "The bacilli are actively motile and possess a single flagellum at one pole", makes it rather uncertain whether his observations were conducted in all cases on species of *B. alvei*. Maassen^(12, 13) found that *B. alvei* is sluggishly motile and is possessed of peritrichous flagella, and the author's observations are in entire agreement with this. Most authors appear to agree as regards their descriptions of the gross cultural characteristics of *B. alvei* (growth on agar, litmus milk, gelatin, colony form, etc.), but reports with reference to the fermentation reactions of this species are by no means consistent. Thus Harrison⁽⁷⁾ reported that acid is formed from lactose, while Maassen⁽¹²⁾ stated that no acid is formed from this sugar. In his work Maassen found that no acid was formed from fructose, galactose or mannitol, while the present work has shown that acid is formed from fructose and from galactose by certain strains. Bergey⁽¹⁾ states that *B. alvei* produces acid from glucose, lactose and sucrose.

The present work indicates that the species hitherto classed as *B. alvei* may be divisible, on the basis of fermentation reactions, into several different species. It would be interesting to determine whether various strains of this organism exhibit serological differences which would permit of their being divided into certain serological groups. It has also been found that another spore-forming organism, apparently differing from others previously described, may take the place of *B. alvei* in European foul brood. These facts may prove to have some bearing on the present confused state of knowledge with reference to the etiology of this disease (Tarr⁽¹⁸⁾). White^(21, 22) believed that European foul brood was caused by a lanceolate-shaped coccus not cultivable on any of the usual media, and he named the organism *B. pluton*. The fact that this organism was never cultured renders any statement to the effect that it exists and, moreover, that it actually causes the disease, open to serious criticism. In fact Burnside⁽³⁾ believes that it is impossible to distinguish between the lanceolate-shaped cells of *Streptococcus apis* (Maassen) and the hypothetical *Bacillus pluton* of White.

Recently Lochhead^(10, 11) discovered that it is possible to stabilise

coccus-shaped variants of *B. alvei* which are morphologically similar to the coccus forms of *Streptococcus apis*, and he suggested that it is possible that *Bacillus alvei* and *Streptococcus apis* are merely stages in the life cycle of a single species. Burnside⁽³⁾, working along similar lines, stated that he had found sporulating rods similar to *Bacillus alvei* in old broth cultures of *Streptococcus apis*, but that nothing was determined concerning their origin. At present, however, it is by no means certain that such changes do occur, and the experimental evidence presented in support of this hypothesis is as yet hardly adequate enough to warrant its adoption.

In the meantime it would seem important to investigate certain other possibilities with a view to settling the remarkable confusion now existing with regard to the etiology of European foul brood. Recent interesting experiments by Shope^(15, 16) and Lewis and Shope⁽⁹⁾ have shown that the disease known as swine influenza results from the combined action of two organisms: a small bacillus closely resembling *Haemophilus influenzae*, and a filterable virus. It is possible that European foul brood offers a rather similar case; a disease caused by the combined action of a filterable virus and certain bacteria (*Streptococcus apis*, *Bacillus alvei*, etc.).

The author⁽¹⁹⁾ found that certain organisms commonly occurring in European foul brood, when fed to young healthy larvae which were subsequently starved, would develop in the host and cause an appearance not unlike that which occurs in actual cases of the disease. On the other hand, when such organisms were fed to larvae which were subsequently attended to normally by the nurse bees they remained healthy and matured normally. Perhaps, then, starvation plays a prominent role in precipitating this disease: it has long been recognised that it is inclined to appear in weak under-nourished stocks in the spring.

SUMMARY.

An unidentified bacillus which appears to replace *B. alvei* in certain cases of European foul brood has been isolated from two different cases of this disease.

Strains of *B. alvei* from various sources exhibit certain differences in their fermentative powers, and it is suggested that these may form a basis for differentiating strains of this organism.

The etiology of European foul brood is discussed.

The author is indebted to those investigators whose generous help in supplying strains of *B. alvei* made this work possible; and his thanks

are due to Dr Schütze of the Lister Institute for useful advice and criticism.

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EXPLANATION OF PLATE XXVIII.

- Fig. 1. Vegetative cells of culture 2 after 18 hours' growth on meat digest agar. Note the variable size of the cells. The spiral structure is a band of broken flagella.
- Fig. 2. Culture 1 showing spore formation in a 3-day-old meat digest agar culture. The cells swell greatly at the time of sporulation, a large, oval spore cyst resulting. The free spores shown are fairly large and are oval.
- Fig. 3. Culture 2 showing spore formation in a 3-day-old meat digest agar culture. The large oval spore cysts are similar to those shown in Fig. 2.
- Fig. 4. Culture 4 showing vegetative cells after 18 hours' growth on meat digest agar. Note the variable size of the cells which are not unlike those shown in Fig. 1 (culture 2).
- Fig. 5. Endospore formation in a 3-day-old egg agar culture of strain 4. Note the arrangement of the spores in chains, and the manner in which the spores are formed in the vegetative cells.
- Fig. 6. Endospore formation in a 3-day-old egg agar culture of strain 8. Spore formation is similar to that shown in Fig. 5 (strain 4).
- Fig. 7. Peritrichous flagella, culture 7.

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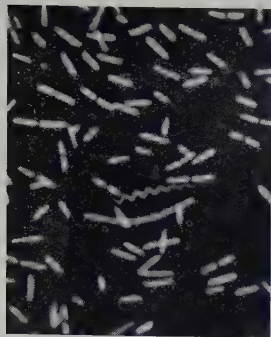


Fig. 1.

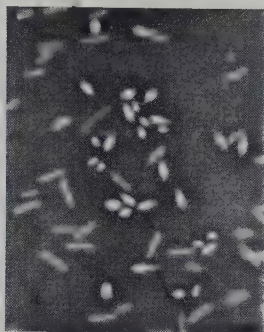


Fig. 2.

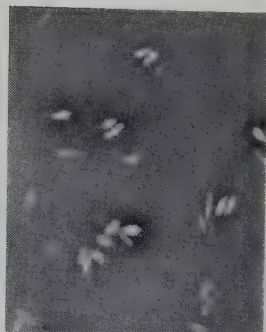


Fig. 3.



Fig. 4.



Fig. 5.



Fig. 6.

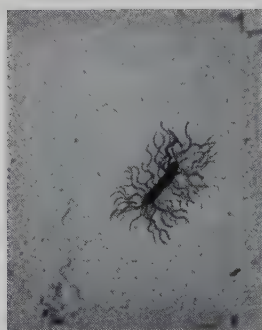


Fig. 7.

FURTHER SEROLOGICAL STUDIES OF PLANT VIRUSES

BY JORGEN M. BIRKELAND.

*(Department of Hygiene and Bacteriology, University of Chicago,
Chicago, Illinois, U.S.A.)*

PLANTS, animals, fungi, and bacteria, by virtue of their chemical constituents, have antigenic specificities characterising their species. These are studied by serologic or immunologic methods which, because of their sensitivity and specificity, have been particularly useful in the identification of bacteria and in the discovery of hidden relationships existing between different strains. The technique has been applied to the study of plant viruses with two ends in view: (1) to determine whether plant viruses are antigenic, that is, capable of stimulating the production of precipitating, complement-fixing, and neutralising antibodies, when injected into rabbits; and, if so, (2) to determine whether serologic specificity can be made use of in their grouping or classification. Much is to be desired regarding the identification of plant viruses which are, as a rule, recognised by their action on a certain plant host, without reference to entity.

Dvorak⁽⁶⁾ in 1927 presented evidence that mosaic disease altered the serologic specificity of the globulin reaction of the cell sap and cytoplasm of the potato plant. Beale^(1, 2) has shown that mosaic-diseased tomato and tobacco plants have in addition to the antigenic components of healthy plants an antigenic substance which is associated with the virus. Several investigators have confirmed and extended these findings. The evidence obtained indicates that the serologic reactions are specific for the virus because:

(1) Virus-diseased plants contain an antigenic substance not present in healthy plants (1, 6, 7, 8).

(2) The serologic titre for the antigenic substance is correlated with the concentration of virus (2).

(3) Virus-containing plant juice (virus antigen) stimulates the production of neutralising antibodies which are specific for the virus⁽⁵⁾.

(4) Purified preparations of several viruses, although not "chemically pure", give positive precipitin reactions only with homologous virus antigens. The reactions are specific. No cross-reactions occur between

tobacco mosaic virus, spot necrosis virus, cucumber mosaic virus, or healthy plant juice (3).

(5) Antisera prepared against plant virus can be absorbed with healthy plant antigen. The absorbed serum contains reactive substances for virus (1, 3).

These data are open to two interpretations: the virus may act as a haptene in not being antigenic itself but in conferring a serologic specificity by virtue of a linkage with plant materials; or, the virus may be antigenic in itself. From the academic point of view it is important to know which of these interpretations is correct; practically it makes little difference. Judging from the results previously reported and from data presented in this paper, it appears that the reactions are specific for the virus and should therefore be of value in differentiating and grouping or classifying them. The present chaotic state in regard to nomenclature is due not so much to inability to differentiate or distinguish viruses as to a lack of means for grouping related viruses.

I. PRECIPITIN REACTIONS WITH VIRUSES PROPAGATED IN SEROLOGICALLY UNRELATED PLANTS.

The greatest difficulty encountered in serologic investigations on plant viruses is that of securing suitable antigens. Since viruses cannot be grown *in vitro*, virus-containing juice from diseased plants must be utilised and this juice contains antigenic material specific for the host. Serum adsorption with healthy plant juices and antigen purification have been used in attempts to eliminate the effect of the common plant antigen and results have indicated that the virus is in itself antigenic. The present experiments attempt to eliminate the effect of common plant antigen by dealing with precipitin reactions of several viruses propagated in serologically unrelated plants. Cucumber mosaic, for instance, has been grown in cucumber and *Nicotiana glutinosa*, serologically distinct plants. It would seem then that if juice from cucumber infected with cucumber mosaic were used to immunise an animal and juice from *N. glutinosa* infected with cucumber mosaic were used as test antigen, cross-reactions occurring must be due to the virus since the common plant antigens have been eliminated.

Experimental procedure.

Viruses used. For this study the following viruses which have a wide host range were chosen: aucuba mosaic virus (tobacco virus 6 (Johnson)), cucumber mosaic virus 1 (Johnson), tobacco mosaic virus 1, and tobacco

ringspot virus (Fromme, Wingard, and Priode). The viruses were grown in greenhouse plants, special care being taken to prevent accidental infection.

Preparation of immunising antigens. Crude juice from healthy or infected plants was obtained by grinding plant leaves in a mortar or putting them through a food chopper, straining the pulp through cheesecloth, allowing it to stand overnight, and then clarifying by passage through filter paper. The clear but highly coloured filtrate thus obtained is hereafter designated as *crude juice*.

(1) *Healthy tobacco plant.* 250 c.c. of crude juice from healthy tobacco plants were treated with glacial metaphosphoric¹ acid to precipitate the proteins. The precipitate, taken up in 50 c.c. of water with sufficient sodium hydroxide to effect a solution with a final pH of 6.4, was used as immunising antigen.

(2) *Aucuba mosaic virus* (tobacco virus 6 (Johnson)) from tomato. 300 c.c. of crude juice from tomato plants infected with a yellow strain of aucuba mosaic virus were treated with 9 c.c. of 1/10 *N* metaphosphoric acid and the precipitate allowed to settle out overnight. The supernatant which contained little virus was discarded and the precipitate, taken up in 50 c.c. of water with sufficient alkali to permit solution, used as immunising antigen.

(3) *Cucumber virus 1* from cucumber. Crude juice from cucumber plants infected with cucumber virus 1 was used as antigen. This was freshly prepared for each injection.

(4) *Cucumber virus 1* from *N. glutinosa*. Crude juice from *N. glutinosa* infected with cucumber virus 1 was used as immunising antigen.

(5) *Tobacco mosaic virus* from tobacco. 500 c.c. of crude juice from tobacco plants infected with tobacco mosaic virus were passed through a Seitz filter and the filtrate discarded. After being washed with 100 c.c. of water, the filter pad was removed from the filter and macerated in 100 c.c. of a basic sodium phosphate buffer. The pulp from the pad was then removed by straining through cheesecloth and the phosphate eluate used as antigen.

¹ The author is indebted to R. K. Schofield, Department of Physical Chemistry, Rothamsted Experimental Station, for suggesting the use of glacial metaphosphoric acid as a precipitant for plant protein. Juice from healthy plants is not a good antigen; that is to say, the titre of the antiserum prepared for it is very low. Since this is apparently due to the low concentration of specific material in the expressed juice it seemed advisable to concentrate the plant antigen by precipitation. Several protein precipitants were tried and glacial metaphosphoric acid finally selected because it did not seem to denature the antigen and could be used with ease.

(6) *Tobacco ringspot virus* from cucumber. Crude juice from cucumber plants infected with tobacco ringspot was freshly prepared for each injection of immunising antigen.

(7) *Tobacco ringspot* from tobacco. 500 c.c. of crude juice from tobacco plants infected with ringspot were purified according to the Seitz filter method described in (5) above.

Preparation of test antigens.

(1) *Aucuba mosaic virus* (tobacco virus 6 (Johnson)). Crude juice from tobacco plants infected with aucuba mosaic virus, green and yellow strains, was used as test antigen.

(2) *Cucumber mosaic virus*. Crude juice from *N. glutinosa* and cucumber plants infected with cucumber-mosaic virus was used as test antigen.

(3) *Tobacco mosaic virus*. Crude juice from tobacco, tomato, and zinnia plants infected with tobacco mosaic virus was passed through a L1 Pasteur-Chamberland candle and the undiluted filtrate used as antigen.

(4) *Tobacco ringspot virus*. Crude juice from tobacco and from cucumber plants infected with tobacco ringspot virus was used as test antigen.

Immunisation of animals. Rabbits weighing from 2400 to 3000 gm. were immunised in duplicate to each of these antigens. Each rabbit was given a total of 36 c.c. of antigen in a series of twelve intraperitoneal injections of 3 c.c. each, a five-day rest period being allowed after each three days of injections. On the sixth day after the last injection the animals were bled aseptically, the blood allowed to clot, and the immune sera collected in sterile tubes.

Precipitin tests. Preliminary precipitin tests were made using (a) increasing dilutions of antigen and constant amounts of antiserum, and (b) increasing dilutions of antiserum and constant amounts of antigen. The practice of diluting the antiserum was the more satisfactory.

Results.

Table I illustrates the method followed in setting up a precipitin test using the antiserum of aucuba mosaic virus, yellow strain, from tomato, against several antigens. The antiserum of aucuba mosaic virus from tomato does not react with the antigen of cucumber mosaic virus from cucumber or with the antigen of tobacco ringspot virus from cucumber. This indicates no serologic relationship between the viruses of aucuba mosaic, ringspot, and cucumber.

Table I.
Precipitin tests with homologous and heterologous antigens.

Antigen virus	Source	Antiserum—aucuba mosaic virus (yellow) from tomato Serum dilutions							
		1-4	1-8	1-16	1-32	1-64	1-128	1-256	1-512
Aucuba (yellow)	Tomato	++	++	++	++	++	++	++	0
Aucuba (yellow)	Zinnia	++	++	++	++	++	++	0	0
Aucuba (yellow)	Tobacco	++	++	++	++	++	++	0	0
Aucuba (green)	Tomato	++	++	++	++	++	++	+	0
Aucuba (green)	Zinnia	++	++	++	++	++	+	0	0
Tobacco mosaic	Tobacco	++	++	++	++	++	+	0	0
Tobacco mosaic	Zinnia	++	++	++	++	+	0	0	0
Tobacco ringspot	Tobacco	++	?	0	0	0	0	0	0
Tobacco ringspot	Cucumber	0	0	0	0	0	0	0	0
Tomato streak	Tomato	++	++	++	++	+	+	0	0
Cucumber mosaic	Cucumber	0	0	0	0	0	0	0	0
Cucumber mosaic	<i>N. glutinosa</i>	++	+	0	0	0	0	0	0
Controls:									
Healthy tobacco		+	0	0	0	0	0	0	0
Healthy tomato		++	+	0	0	0	0	0	0
Healthy <i>N. glutinosa</i>		+	0	0	0	0	0	0	0
Healthy zinnia		0	0	0	0	0	0	0	0

Antigen controls with 0.85 % NaCl and with normal serum were negative.

? = indecisive, 0 = no reaction, + = faint precipitate, ++ = slight but definite precipitate, +++ = moderate precipitate, ++++ = heavy precipitate.

Table II.
Precipitin tests with homologous and heterologous antigens.

Virus used in producing antisera ...		Cucumber virus 1		Cucumber virus 1		Cucumber virus 1		Ringspot virus		Ringspot virus (attenuated)		Tobacco virus 1		Aucuba mosaic (green)		Aucuba mosaic (yellow)	
Source
Test antigens		Virus		Source		Cucumber		N. glutinosa		Cucumber		Tobacco		Tobacco		Tomato	
Cucumber virus 1				Cucumber		+++ (1:16)	+++ (1:16)	+++ (1:16)	0	0	0	0	0	0	0	0	0
Cucumber virus 1				N. glutinosa		+++ (1:16)	+++ (1:32)	+++ (1:16)	0	0	0	0	0	0	0	0	0
Ringspot virus				Cucumber		0	0	+++ (1:16)	+++ (1:32)	+++ (1:32)	+++ (1:32)	+++ (1:32)	+++ (1:32)	+++ (1:32)	+++ (1:32)	+++ (1:32)	+++ (1:32)
Ringspot virus				Tobacco		0	0	+++ (1:16)	+++ (1:32)	+++ (1:32)	+++ (1:32)	+++ (1:32)	+++ (1:32)	+++ (1:32)	+++ (1:32)	+++ (1:32)	+++ (1:32)
Tobacco virus 1				Tobacco		0	0	+++ (1:16)	+++ (1:32)	+++ (1:32)	+++ (1:32)	+++ (1:32)	+++ (1:32)	+++ (1:32)	+++ (1:32)	+++ (1:32)	+++ (1:32)
Tobacco virus 1				Zinnia		0	0	0	0	0	0	+++ (1:128)	+++ (1:128)	+++ (1:128)	+++ (1:128)	+++ (1:128)	+++ (1:128)
Aucuba mosaic (green)				Zinnia		0	0	0	0	0	0	+++ (1:32)	+++ (1:32)	+++ (1:32)	+++ (1:32)	+++ (1:32)	+++ (1:32)
Aucuba mosaic (yellow)				Tobacco		0	0	0	0	0	0	+++ (1:128)	+++ (1:128)	+++ (1:128)	+++ (1:128)	+++ (1:128)	+++ (1:128)
Aucuba mosaic (yellow)				Tomato		0	0	0	0	0	0	+++ (1:128)	+++ (1:128)	+++ (1:128)	+++ (1:128)	+++ (1:128)	+++ (1:128)
Aucuba mosaic (yellow)				Zinnia		0	0	0	0	0	0	+++ (1:64)	+++ (1:64)	+++ (1:64)	+++ (1:64)	+++ (1:64)	+++ (1:64)
Tomato streak virus				Tomato		0	0	0	0	0	0	+++ (1:64)	+++ (1:64)	+++ (1:64)	+++ (1:64)	+++ (1:64)	+++ (1:64)
Controls:																	
Healthy cucumber						0	0	0	0	0	0	0	0	0	0	0	0
Healthy N. glutinosa						0	0	0	0	0	0	0	0	0	0	0	0
Healthy tobacco						0	0	0	0	0	0	0	0	0	0	0	0
Healthy tomato						0	0	0	0	0	0	0	0	0	0	0	0
Healthy zinnia						0	0	0	0	0	0	0	0	0	0	0	0

+++ indicates a heavy precipitate.

The figures given in parentheses under these +++ reactions refer to the highest dilution of antiserum in which complete precipitation occurred. The 0's indicate not reaction at an antiserum dilution of 1:8.

The antiserum of aucuba mosaic virus from tomato reacts with the antigen of aucuba mosaic virus, green or yellow strains, from zinnia and with tobacco mosaic virus from zinnia. Since zinnia does not show a serologic relationship to either tobacco or tomato, the reaction must be attributed to the virus and indicates the close relationship of aucuba mosaic virus, green and yellow strains, and tobacco mosaic virus.

The differences in titres obtained are not significant, since the concentration of aucuba mosaic virus in zinnia was much greater than that of tobacco mosaic virus in zinnia. In other tests conducted the antiserum of tobacco mosaic virus from tobacco reacted in higher titre with aucuba mosaic virus from zinnia than with tobacco mosaic virus from zinnia (see Table II).

Table II summarises the results obtained for each antiserum used against each antigen. As previously noted, the viruses have been propagated in both serologically related and unrelated plants. It can be seen that the viruses listed fall into three distinct categories: (1) cucumber mosaic virus; (2) tobacco mosaic virus, aucuba mosaic virus, and, probably, tomato streak virus; and (3) tobacco ringspot virus. Viruses from each category show no cross-precipitin reactions and can therefore be considered serologically unrelated.

Reciprocal precipitin tests were not run with tomato streak virus, but the titres obtained with tobacco mosaic virus antiserum and aucuba mosaic virus antiserum indicate a close relationship. It may be that as the technique of preparing and standardising antigens is improved a serologic difference will be found between these viruses and even between different strains of these viruses.

II. AN ATTEMPT TO ISOLATE A SPECIFIC SOLUBLE SUBSTANCE.

The chemical nature of plant viruses is unknown. Although they show many of the characteristics of proteins, it has not been established definitely that they are protein in nature. Proof that they are antigenic would be good presumptive evidence on that point, since, in general, only proteins act as antigens. To be sure many substances, such as carbohydrates¹ and lipins, can confer serologic specificity to protein molecules when linked to them; that is, they can function as haptenes and can react specifically with appropriate antisera in the absence of proteins.

The demonstration by Burnet⁽⁴⁾ that a specific soluble substance is associated with bacteriophage and the importance of the specific soluble

¹ In a few cases polysaccharides have been shown to be antigenic.

substance in the serologic specificity of many bacteria and some fungi has suggested the possibility of similar elements being involved in the specificity of plant viruses. Accordingly a few preliminary attempts were made to isolate a specific soluble substance from the juice of virus-diseased plants.

Experimental procedure.

Viruses used. The following viruses were propagated in the plants indicated: aucuba mosaic (green strain), tobacco; aucuba mosaic (yellow strain), tobacco; tobacco mosaic, tobacco; tobacco ringspot virus, cucumber; and cucumber mosaic, *N. glutinosa*. Healthy plants were used as controls.

Method. The procedure, in brief, consisted in precipitating the carbohydrates from 4 litres of crude juice with 1.5 volumes of 95 per cent. ethyl alcohol, discarding the supernatant, resuspending the precipitate in 1000 c.c. of water, again precipitating the carbohydrate with 1.5 volumes of alcohol, and so on.

After a fourth resuspension a portion of the solution was treated with sufficient saturated trichloroacetic acid to bring about a concentration of from 3 to 10 per cent. No precipitant appeared, even after standing overnight, thus indicating that protein, if present, was in exceedingly minute quantity.

In all, the carbohydrates were precipitated and resuspended eight times. The final precipitate was washed three times with absolute alcohol, once with ether, and then dried at 37°C. The amount yielded from the 4000 c.c. of crude juice was approximately 0.013 gm. and was not readily soluble in water. It contained traces of nitrogen, and on hydrolysis yielded glucose.

Serologic tests. This precipitate was tested for serologic properties by mixing with antiserum prepared against the virus. Dilutions of 1/200 to 1/200,000 of the carbohydrate were used.

Results. No precipitate appeared with antiserum nor was there any evidence of a "blocking" effect on the virus-antiserum reaction.

The juice from healthy plants, similarly treated, yielded even less precipitate.

While these experiments do not prove that a specific soluble substance is not associated with the virus, they do indicate that, according to the methods employed in this experiment, it could not be detected.

SUMMARY.

Precipitin reactions with several plant viruses propagated in serologically unrelated hosts offer additional evidence that the virus is, in itself, antigenic.

Reciprocal precipitin tests show that the viruses of cucumber mosaic, tobacco ringspot, and tobacco mosaic are serologically distinct; whereas tobacco mosaic virus, aucuba mosaic virus (green and yellow strains), and probably tomato streak virus remain serologically indistinguishable.

No soluble specific substance was isolated from the juice of virus diseased plants or of healthy tomato by the methods employed.

This study was conducted at the Rothamsted Experimental Station, Harpenden, Hertfordshire, England, during the tenure of a National Research Council Fellowship in Biological Science. The writer wishes to thank Dr J. Henderson Smith for providing the facilities for the work and for his helpful interest in it.

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THE CHLOROTIC DISEASE OF THE HOP

IV. TRANSMISSION BY SEED

BY PROF. E. S. SALMON AND W. M. WARE, D.Sc.

(Mycological Department, South-Eastern Agricultural College, Wye, Kent.)

(With Plate XXIX.)

IN a previous number of this *Journal* ⁽¹⁾ the chlorotic disease of the hop, a virus disease, was named and described. Experiments have also been detailed ^(1, 2, 3) which show how it can be transmitted artificially to healthy plants. It has been found, *inter alia*, that the simple process of rubbing the leaf of a healthy plant with the juice of macerated diseased leaves communicates the disease. In most instances it has been found that an infected plant does not show the symptoms until the season following that in which the inoculation has been made ^(2, 3).

The present paper shows that the virus can be transmitted naturally through the "seed".

In the autumn of 1932 "seed" was collected from cones produced from "open" flowers of three hop plants affected with the chlorotic disease (Ref. Nos. AA 4, AA 5, and OR 104), growing in the Experimental Hop Garden at the South-Eastern Agricultural College, Wye, Kent. The origin of the diseased plants was as follows: AA 4 and AA 5 were chlorotic plants, probably of the Fuggle variety, sent in 1927 by a hop-grower farming near Tenbury, Worcestershire. OR 104 was of the Tolhurst variety and had become chlorotic in 1932 as a result of having been grafted, in the spring of 1931, with scions from a naturally infected plant, probably of the Fuggle variety (Ref. No. C 20), which had the same origin as AA 4 and AA 5 above.

The "seeds" were sown early in 1933 in seed boxes in the usual manner in an unheated glasshouse in which no chlorotic hop plants were kept, and the seedlings, when sufficiently large, were transferred singly to pots. Chlorotic symptoms were first observed in the pot plants in May 1933. On June 8th, 1933, when the plants, 228 in number, were from 1½ to 4½ ft. high, they were closely examined and could be classified as follows:

(1) *Affected with chlorotic disease. Class 1.* Some of the leaves distorted and domed, as illustrated in Plate XXIX, fig. 1, commonly accompanied with chlorotic blotching on leaves not distorted. As is usually the case with the present disease, the stems were growing away

from the disease and forming three or four pairs of normal leaves above the deformed pairs. There were twenty-three plants in this class.

Class 2. Chlorotic blotching present at the margin of one or two leaves only and no distortion of the lamina. There were five plants in this class.

(2) *Healthy.* 200 plants.

Thus, in 1933, twenty-eight plants of the 228 seedlings (12.3 per cent.) showed the disease.¹

All the diseased plants were destroyed in June 1933 and the healthy plants were retained for observation in 1934.

On March 13th, 1934, four plants, all of the dwarf (non-climbing) type (see (4)), showed chlorotic markings, with two or three deformed leaves on each. On April 16th, 1934, five more plants of the dwarf type were showing chlorotic symptoms; these included chlorotic blotching and domed deformity of the lamina in some cases.

On April 16th, 1934, also, four of the climbing plants showed chlorotic blotches; three of these exhibited as well the domed deformity and one only a slight deformity of the leaf. On May 1st, 1934, the next examination of the seedlings was made and the disease found on a further seventeen plants of which twelve were in class 1 (see above) and five in class 2. On May 18th the final examination was made and three more plants affected with the disease were found, one in class 1 and two in class 2.

The total number of diseased plants in 1934 was therefore thirty-three. Of the 200 plants saved from 1933, four plants had to be destroyed owing to the presence of downy mildew before it could be known whether they were affected with chlorotic disease or not. The total number therefore was 196, of which thirty-three plants, or 16.8 per cent., showed the disease. Taking all the diseased plants in the two years, sixty-one (26.8 per cent.) of the original 228 showed the chlorotic symptoms.

The various ratios of the transmission of the disease in the seedlings obtained from the three parent plants are as follows:

Parent plant	Chlorotic		Healthy	Percentage chlorotic
	1933	1934		
AA 4 (probably Fuggle)	12	15 ⁺	55 ⁺⁺⁺	32.93
AA 5 (probably Fuggle)	15	14 ⁺⁺	52 ⁺⁺⁺⁺	35.80
OR 104 (Tolhurst)	1	4	56 ⁺⁺⁺⁺⁺	8.19
	28	33	163	

⁺ including 2 dwarfs, ⁺⁺ including 7 dwarfs, ⁺⁺⁺ including 7 dwarfs,
⁺⁺⁺⁺ including 11 dwarfs, ⁺⁺⁺⁺⁺ including 1 dwarf.

¹ Of some thousands of hop seedlings raised in 1933 and 1934 from healthy parents, in contiguous boxes in the same glasshouse, none showed chlorotic disease.

According to Dr Kenneth Smith⁽⁵⁾, transmission of a virus disease by seed is not of common occurrence, but "there are several authentic cases, the best known being mosaic of bean, *Phaseolus vulgaris*, where as high as 50 per cent. infection may sometimes develop". Although, in the present instance, insect-proof or other controlled conditions could not be maintained, in the authors' opinion there is no doubt that the chlorosis was transmitted through the seed of the three hop plants used and not through any external means.

SUMMARY.

In 228 seedlings raised from hop plants affected with chlorotic disease, transmission of the disease occurred in twenty-eight plants (12.3 per cent.) in the first year and, of the remaining 196 healthy seedlings,¹ thirty-three plants (16.8 per cent.) showed the disease in the second year. Thus, of the 228 seedlings raised, sixty-one (26.8 per cent.) eventually showed chlorotic symptoms.

In the authors' opinion, transmission of the disease was through the seed.

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EXPLANATION OF PLATE XXIX.

Fig. 1. Chlorotic disease of the hop transmitted through the seed. A seedling, derived from a naturally chlorotic plant (Ref. No. AA5), showing the symptoms in the first few months of its existence. Three pairs of leaves are domed, distorted and chlorotic. One large leaf (right) shows a chlorotic area on each basal lobe of the cordate lamina. Seed sown March 2nd, 1933. Photographed May 28th, 1933. ($\frac{2}{3}$ nat. size.)

¹ Four of the original 200 healthy seedlings in 1934 had to be destroyed owing to attacks of Downy Mildew.

(Received April 15th, 1935.)



Fig. 1.

SALMON AND WARE.—THE CHLOROTIC DISEASE OF THE HOP (pp. 728-730).

A NEW VIRUS DISEASE OF THE TOMATO

By KENNETH M. SMITH, D.Sc., Ph.D.

(*Potato Virus Research Station, School of Agriculture, Cambridge.*)

(With Plates XXX-XXXII.)

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INTRODUCTORY.

AMONG some virus-diseased material received for examination were tomato plants which exhibited unfamiliar symptoms. Inoculation from these tomatoes to a series of differential hosts revealed the fact that a new virus was concerned in the production of the disease(3). In this paper a description is given of the chief characteristics of the virus and the symptoms it produces on a comprehensive series of host plants.

SYMPTOMATOLOGY AND HOST RANGE.

The virus is sap-transmissible by the ordinary methods of inoculation and is quite infectious. One of the characteristics is the extremely short incubation period of the virus in certain host plants.

Tomato.

Local symptoms develop on the inoculated leaf about 5 days after inoculation at a mean daily temperature of 60-70° F. These local symptoms usually consist of lesions either in the form of rings (Plate XXX, fig. 3) or circular necrotic patches. A little later the inoculated leaves become pale yellow on which patches of green remain outstanding; frequently these leaves drop off. The development of the disease from this

point seems to depend somewhat on the age of the tomato plant. In small plants, 3-4 in. high, there develops a general necrosis of the leaves together with yellow and purple coloration. In young tomato plants with soft sappy stems a gross necrotic lesion frequently develops at soil level and, passing downwards, involves the root system, bringing about the rapid death of the whole plant (Plate XXX, fig. 2). It is possible, however, that the actual lesion in the tomato may be due to secondary causes, although the virus seems to be the sole cause of similar lesions in the stems of affected *Datura* plants.

In older tomato plants the progress of the disease is less rapid and the stem lesion rarely develops; the end result, however, is almost equally destructive. After the development of local lesions on the leaves there is an almost complete cessation of growth, the youngest leaves frequently become pale yellow in colour and twist over, sometimes being completely reversed. Occasionally a necrosis develops which kills the growing points. The lower leaves then become chlorotic and usually undergo a colour change to yellow or purple which is highly characteristic. A common symptom is the appearance of concentric rings of bright yellow or purple (Plate XXX, fig. 4) or, alternatively, necrotic lines of a purple colour develop along the veins on the yellow background of the leaf. The lower leaves finally shrivel or become completely chlorotic and drop off.

The symptoms on the ripe fruit consist of a characteristic mottling or blotching of pale spots or ring-like marks on a darker background (Plate XXX, fig. 5). The green fruits appear normal though the virus is present in them.

The outstanding characteristics of the disease in tomatoes are cessation of growth, yellowing and purpling of the lower leaves, and chlorosis, distortion or proliferation of the youngest leaves.

Tobacco, White Burley.

Local lesions develop on the inoculated leaves 3 days after inoculation at a mean daily temperature of 60-70° F. When they first develop the lesions are small, red in colour and are surrounded by a yellowish halo; they then rapidly dry out and become paper white in colour (Plate XXXI, fig. 4). The lesions do not increase much in size as is the case with the lesions formed on *Nicotiana glutinosa* and cowpea. As a rule no further infection ensues and the plant remains healthy. Occasionally, however, in about 10 per cent. of the inoculated plants there is a slight further development of the disease. A few scattered necrotic

lesions may develop on the uninoculated leaves about 14 days after the appearance of the local symptoms. Experiment showed that these lesions contain the virus but that there was no virus in the intervening tissue. There is no mosaic mottling in affected tobacco plants and no systemic invasion in the usual sense of that term.

Nicotiana glutinosa.

On this species small round lesions develop on the inoculated leaves 48 hours after inoculation. The lesions gradually increase in size, becoming quite large, sometimes measuring 5 mm. in diameter; at this stage the lesions have a dark red edge and a pale centre (Plate XXXII, fig. 2). As in the case of affected tobacco plants there is usually no further development of the disease; occasionally, however, a few scattered necrotic lesions develop on the younger uninoculated leaves (Plate XXXII, fig. 3). The virus is present in these lesions but not in the intervening tissue.

Nicotiana langsdorffii.

The behaviour of the virus on this plant resembles that on tobacco and the local lesions are similar. The development of systemic lesions has not been observed on *N. langsdorffii*.

Datura stramonium.

The symptoms induced by the virus in *Datura* are quite characteristic, and in consequence the plant is a valuable differential host. Circular or dendritic yellow spots appear locally about 5 days after inoculation and systemic invasion develops normally. The disease is a very severe one, and the main characteristics are the crinkling and blistering of the leaves and the very marked ochre yellow and green mottle. Affected *Datura* plants exhibit a very bold yellow and green variegation, and the normal shape of the leaves is lost. Such *Datura* plants are only with difficulty recognisable (Plate XXXII, fig. 4). Occasionally a stem lesion develops, causing the plant to assume a distorted habit of growth.

Potato, var. Arran Victory.

Local lesions are produced with difficulty on the inoculated leaves of this variety of potato. No systemic spread of the virus has been observed.

Cowpea (Vigna sinensis).

The reaction of the cowpea to this virus is very characteristic and serves to distinguish it from other viruses commonly infecting the tomato plant. Small lesions develop on the inoculated leaves 3-4 days after

inoculation. These lesions, at first pale, rapidly turn red and increase in size; the appearance of the lesions a week or two after their first appearance is shown in Plate XXXI, fig. 2. Note the deep red edge and the pale centre. Systemic spread of the virus in the cowpea has not been observed and symptoms appear to be confined to the inoculated leaves.

Miscellaneous host plants.

No systematic study of the host range of the virus outside the Solanaceae has been attempted, but infections have been obtained on mimulus (Scrophulariaceae), aster and zinnia (Compositae). In all these plants local lesions only resulted and there appeared to be no further development of the disease.

PHYSICAL PROPERTIES OF THE VIRUS.

(1) *Resistance to ageing in vitro.*

The virus loses its viability fairly rapidly when stored in extracted sap at room temperatures. The virus concentration, as measured by local lesion counts on *Nicotiana glutinosa* or cowpea, showed a steady fall during the period the virus retained its viability. For example, a virus-sap suspension which gave numerous local lesions on the first day, gave two lesions only on the 25th day and no infection at all on the 33rd day. The virus showed no apparent reduction in concentration after storage for 28 days at $+1^{\circ}\text{C}$.

Further data on the resistance to ageing of this virus are given in Table I.

Table I.

Longevity in vitro of the tomato virus.

Experiment No.	Temperature	Ageing in days	No. of plants infected out of three inoculated*	Average no. of lesions per plant
1	Room temp.	4	3	10-15
		10	3	5-6
		14	1	1
		19	Nil	Nil
		33	Nil	Nil
2	"	4	3	10
		14	2	6-10
		19	2	2
		25	1	2
		33	Nil	Nil
3	"	14	3	1-2
4	"	22	1	1
5	$+1^{\circ}\text{C}$.	28	3	20

* The average number of lesions per plant for the control inoculations is taken as 25. The experimental plants used were *N. glutinosa* and cowpea.

(2) *Thermal death-point.*

In measuring the thermal death-point 1-2 c.c. of crude expressed virus sap in thin-walled test-tubes were used. The tubes were immersed in a water bath for 10 min. at the required temperature and then plunged into cold water. The data are given in Table II.

Table II.

Thermal death-point.

Temperature °C.	No. of tests made at each temperature	Total no. of plants inoculated	No. of plants infected	Average no. of lesions* per plant
50	1	5	5	10-15
60	1	5	5	6
70	1	5	2	3
75	4	20	3	2
78	2	10	4	2
80	3	15	Nil	Nil

* The average number of lesions per plant for the control inoculations is taken as 25. The experimental plants used were *N. glutinosa* and cowpea.

(3) *Resistance to alcohol.*

The results of the experiments on the resistance of the virus to alcohol are given in Table III.

Table III.

Resistance of the tomato virus to alcohol.

Experiment No.	Time in alcohol hours	Strength of alcohol %	No. of plants infected out of 4 inoculated	Average no. of lesions per plant
1	4	65	4	30-40
	4	70	4	20
	4	75	4	20
	4	80	4	10
2	24	20	4	10
	24	40	4	5-10
	24	50	4	5-10
	24	80	2	2-3
3	24	50	4	10
	24	60	4	5
	24	70	3	2
	24	75	Nil	Nil
4	24	80	1	1
	24	80	2	5
	24	85	2	5
	24	90	1	2
5	24	95	1	1
	24	80	1	1
	24	85	1	1
	24	90	—	—
6	24	95	—	—
	24	85	2	2
	24	90	—	—
	24	90	—	—

Experiments 8:

(4) *Dilution end-point.*

Although the amount of dilution a given virus will stand naturally depends on its initial concentration, there exists a considerable difference in the reaction of the various viruses to this test under uniform conditions. With the present tomato virus, young leaves of tomato, *Datura*, and *Nicotiana glutinosa* were employed, only recently infected plants being used. Dilutions with distilled water up to one in a million were tested, using crude expressed virus sap which had been passed through muslin to remove the larger particles. Infection was rarely obtained at dilutions greater than 1 : 10,000 with the crude expressed sap. With sand-and-pulp filtrates, infections were only obtained four times out of sixteen tests at this dilution (see Table IV). In view of the ultrafiltration results, this low dilution end-point is rather unexpected and seems to indicate that adsorption of the virus on the membranes must be very small.

(5) *Desiccation.*

The virus appears unable to withstand drying. A series of experiments was carried out in which inoculations were made at intervals to cowpeas and *N. glutinosa*, using, as a source of inoculum, inoculated leaves of *N. glutinosa* which had been allowed to dry in envelopes at room temperature. The number of local lesions given by these leaves gradually fell off until a single lesion only was produced on three plants of *N. glutinosa* after the infected leaves, used as a source of inoculum, had been kept for 30 days.

Since it is difficult to be certain that the infected leaves from which inoculations are to be made are completely desiccated, tests were made with the virus collected and dried on an impermeable membrane, and similarly with a virus suspension collected and dried on Kieselguhr. From both these tests negative results were obtained.

ULTRAFILTRATION STUDIES.

Experiments have been carried out on the ultrafiltration of the virus through Gradocol membranes(1). It has been found that the virus is easily filterable and is of very small size.

The virus sap was used undiluted and was clarified for ultrafiltration by passage of a Kieselguhr bed. In nearly every case the virus concentration of the Kieselguhr filtrate was ascertained by dilution tests before passage through the membranes.

The data of the ultrafiltration studies are given in Table IV; it will be seen that the filtration end-point is about 0.05 μ . According to

Elford's method of calculation (2), the filtration end-point of 0.05μ gives to the virus an approximate particle size of $17-25m\mu$.

Table IV.
Data of ultrafiltration studies.

Membrane thickness mm.	A.P.D. of membrane μ	Titre of Kieselguhr filtrate	No. of plants infected out of 5 inoculated		No. of lesions per experimental plant
			Control	Experi- mental	
0.16	0.35	Not tested	5	5	10-20
0.165	0.16	Not tested	5	5	10
0.20	0.11	1: 10,000	5	5	5-10
0.187	0.10	1: 1,000	5	3	5
0.20	0.098	1: 10,000	5	5	6-8
0.20	0.098	1: 1,000	5	4	4
0.23	0.086	1: 1,000	5	3	2-3
0.23	0.086	1: 1,000	5	5	5-10
0.215	0.074	1: 1,000	5	3	5
0.215	0.074	1: 1,000	5	5	10
0.215	0.074	1: 1,000	5	3	1-2
0.175	0.063	1: 1,000	5	1	2
0.160	0.060	1: 10,000	5	1	3
0.160	0.060	1: 1,000	5	3	3
0.160	0.060	1: 1,000	5	1	2
0.185	0.050	1: 10,000	5	1	1
0.185	0.050	1: 1,000	5	Nil	Nil
0.185	0.050	1: 1,000	5	Nil	Nil

The average number of lesions for the control plants is taken as 20-30 per plant.
A.P.D. = average pore diameter. Inoculations made to cowpea in each case.

TRANSMISSION TESTS.

By seed. One hundred plants were raised from seed collected from the mottled fruits shown in Plate XXX, fig. 5. All these plants were healthy. This test seems to indicate that seed transmission, if it occurs, is not frequent.

By insects. The insect transmission studies are still in progress, but tests with the aphid, *Myzus persicae*, have given consistently negative results.

IMMUNITY TESTS.

Recent work has shown that infection with one virus confers upon a plant an immunity to further infection with similar viruses or virus strains. Some experiments on these lines were performed to ascertain whether any such affinity existed between the new tomato virus and the following viruses, two of which commonly affect the tomato plant in this country.

Tomato mosaic (tobacco virus 1).

Eighteen small tomato plants were inoculated with the virus of tomato mosaic and, as soon as systemic infection was established, twelve of these plants were inoculated with the new tomato virus. The other six plants were left as controls. After 5-7 days the twelve tomato plants mentioned above commenced to show the local lesions characteristic of infection with the new virus. The disease then followed its normal course and there appeared to be no increased reaction due to the presence of the mosaic virus as is the case with "glasshouse streak".

Tomato spotted wilt.

Three small tomato plants in the "bronzing" stage of spotted wilt infection were inoculated with the new virus. After the usual incubation period the second disease developed, although local lesions on the bronzed leaves were not observed. Inoculations from these tomato plants back to differential hosts showed that the second virus had entered the tomato plants already systemically infected with the virus of spotted wilt.

Tobacco ringspot (Wingard (4)).

Three White Burley tobacco plants systemically infected with tobacco ringspot were reinoculated with the tomato virus. The usual local lesions characteristic of this virus developed without any further spread.

An undescribed tobacco ringspot.

Immunity tests were also made using White Burley tobacco plants infected with an undescribed tobacco ringspot virus collected by the writer at Bergerac in south-western France. As with the foregoing ringspot virus no protection against the entry of the tomato virus was afforded.

From these tests it seems clear that no immunity from infection with the new tomato virus is conferred by previous infection with some of the common viruses infecting tobacco and tomato. By analogy therefore it seems that this new virus is entirely distinct from these other viruses, and this is borne out by comparisons of their respective symptoms and physical properties.

DIFFERENTIATION OF THE VIRUS FROM OTHER VIRUSES
COMMONLY AFFECTING THE TOMATO.

Tomato mosaic (tobacco virus 1).

The virus can readily be distinguished from tobacco virus 1 by a variety of tests. Its symptomatology on different host plants is quite distinct. Thus, tobacco virus 1 does not infect cowpea (*Vigna sinensis*),

it is invariably systemic with a mosaic mottling in tobacco, and produces only a mild mottling in tomato. The new virus, on the other hand, produces characteristic lesions on cowpea, is not usually systemic in, and does not produce mottling in, tobacco, and the local and systemic symptoms produced in tomato are highly characteristic. The physical properties of the two viruses are also on the whole different. The resistance to ageing *in vitro* of the new virus can be measured in weeks, while tobacco virus 1 will remain viable for periods of years. The respective dilution end-points are also very different.

There are one or two points of resemblance between the new virus and tobacco virus 1, the type of lesion produced in *Nicotiana glutinosa* by the former virus resembles in its early stages the lesions characteristic of tobacco virus 1 (Plate XXXII, fig. 1). The second point is the tolerance of both viruses to high concentrations of alcohol, and thirdly both viruses have a very small particle size as measured by ultrafiltration. The size of the viruses arrived at by ultrafiltration in plant sap is about $20\text{m}\mu$; if anything, the tomato virus is slightly smaller.

Tomato streak virus 1 (tomato stripe).

There is little difficulty in distinguishing between the new virus and tomato streak virus 1 by the respective symptoms on various host plants. While it is true that both viruses may produce stem lesions on tomato, the lesion produced by the former is usually at or below soil level, involving the root system without longitudinal "striping" of the stem, while the lesions characteristic of tomato streak develop higher up the stem. In addition there are the rings and colour changes produced in tomato by the new virus.

On White Burley tobacco, both viruses produce necrotic lesions, but those formed by tomato streak virus 1 are followed either by systemic mottling or pronounced necrosis. The lesions do not dry out and become white as in the case of the new virus (Plate XXXI, fig. 4).

Tomato streak virus 1 does not infect cowpea, and this alone is sufficient to differentiate between the two (Plate XXXI, figs. 1 and 2).

Tomato spotted wilt.

The virus of spotted wilt has a few points in common with the new virus, notably in the tendency of both to form concentric rings on certain host plants. There is, however, a wide divergency between the two in many aspects of their behaviour. There is no bronzing of the leaves of tomatoes with the new virus, and the symptoms produced on White

Burley tobacco, *N. glutinosa* and cowpea (*Vigna sinensis*) are quite distinct. Moreover, the respective physical properties are very different, while the virus of spotted wilt will only remain viable in expressed sap for 4-6 hours at room temperatures, the new virus retains its infective power for periods of weeks under similar conditions. Furthermore, it is a very small virus and is easily filtered while the virus of tomato spotted wilt is only filtered with very great difficulty.

SUMMARY.

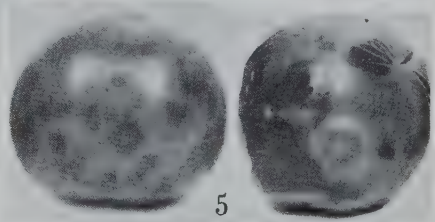
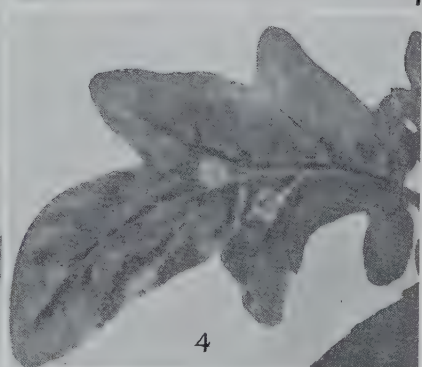
A new virus disease of the tomato plant is described. An account is given of the symptoms produced on a variety of host plants, mostly in the Solanaceae. Some of the physical properties of the virus have been investigated and its particle size measured by ultrafiltration through Gradocol membranes. The size as measured by filtration in plant sap is approximately 17-25 μ . The virus is compared with other viruses commonly affecting tomato and tobacco and methods of differentiating them are discussed.

Grateful acknowledgments are due to Prof. D. Keilin, F.R.S., who afforded the facilities for the ultrafiltration studies in the Molteno Institute, and to Mr J. P. Doncaster for his assistance in this work and for taking the photographs. The writer is also indebted to Mr Lawrence Ogilvie who first sent the virus-affected plants.

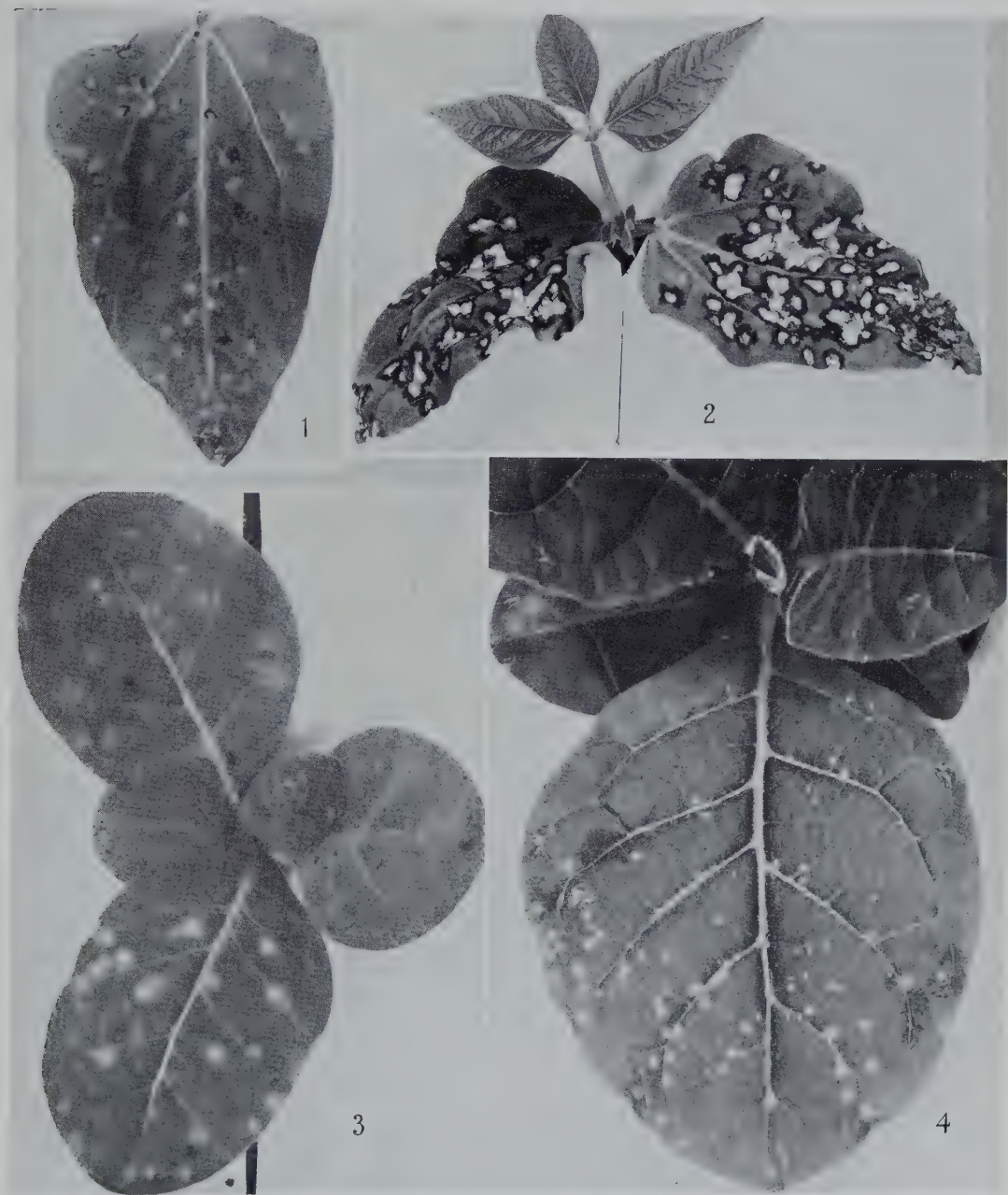
The experimental plants used in this study were grown in a glass-house provided with a grant from the Royal Society Grants Committee to whom the writer's best thanks are due.

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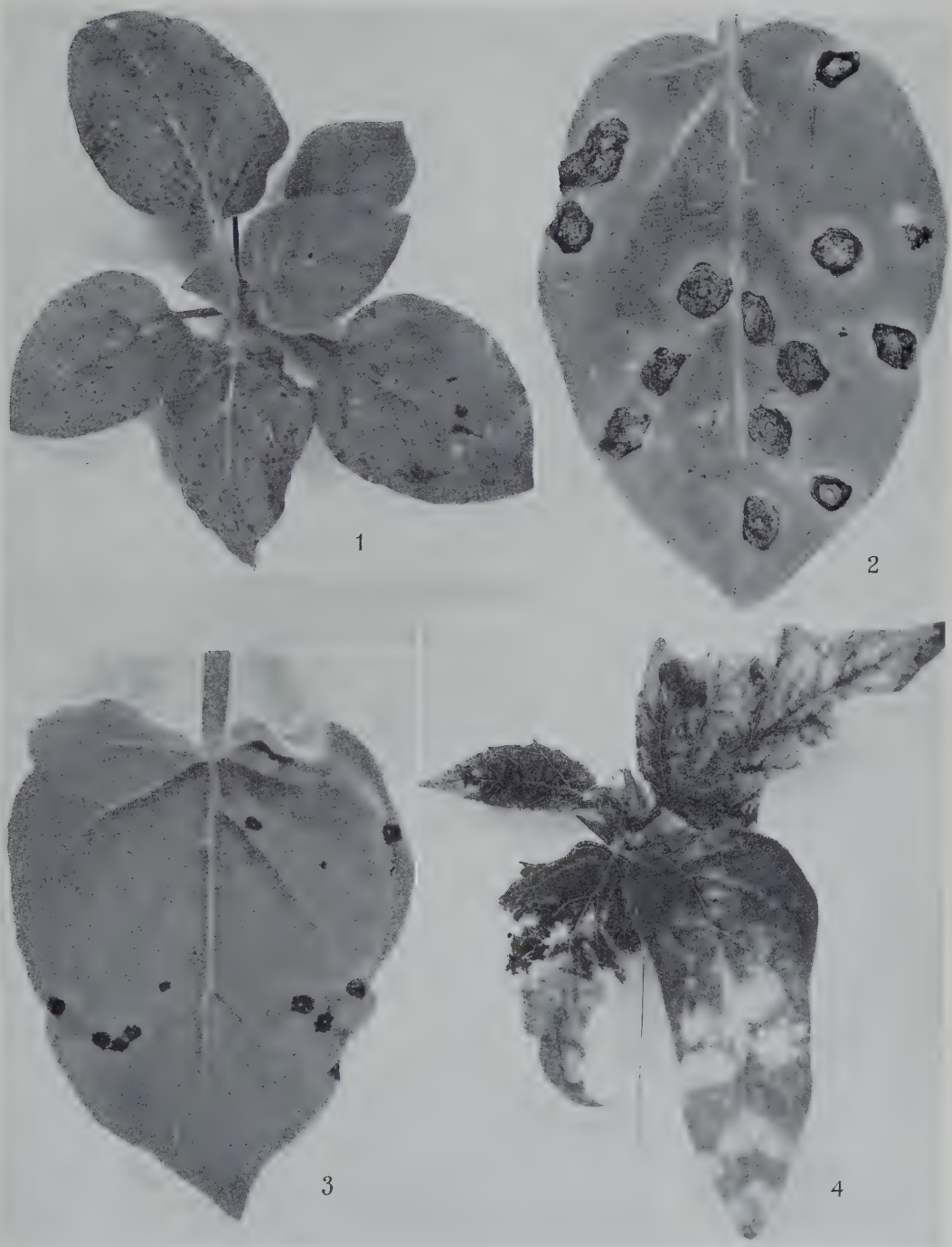
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SMITH.—A NEW VIRUS DISEASE OF THE TOMATO (pp. 731-741).



SMITH.—A NEW VIRUS DISEASE OF THE TOMATO (pp. 731-741).



SMITH.—A NEW VIRUS DISEASE OF THE TOMATO (pp. 731-741).

EXPLANATION OF PLATES XXX—XXXII.

PLATE XXX.

- Fig. 1. Tomato plant showing the symptoms of systemic infection.
Fig. 2. Young tomato plant showing collapse of lower leaves and lesion at soil level.
Fig. 3. Local lesions in the form of single rings on the inoculated leaves of a tomato plant.
Fig. 4. Concentric rings which have developed after systemic infection, in this case the rings are of a bright yellow colour.
Fig. 5. Tomato fruits showing the mottling characteristic of the disease.

PLATE XXXI.

- Fig. 1. Local lesions on cowpea leaf, 5 days after inoculation. Note that the lesions which appeared first have become dark red, later lesions still pale in colour.
Fig. 2. Local lesions on cowpea about 14 days after inoculation. Note that the young leaves are free of the virus.
Fig. 3. Local lesions on White Burley tobacco, 3 days after inoculation.
Fig. 4. The same plant 3 weeks later. Note that the lesions have dried and that there has been no systemic spread of the virus.

PLATE XXXII.

- Fig. 1. Local lesions on *N. glutinosa*, 48 hours after inoculation.
Fig. 2. Local lesions on *N. glutinosa*, 3 weeks after inoculation.
Fig. 3. Systemic lesions on an uninoculated leaf of *N. glutinosa*, these lesions have only developed in a small number of cases.
Fig. 4. Symptoms produced on *Datura stramonium*: these consist of a bright yellow and green mottle, much distortion of the leaves and the development of dark green blisters.

APPENDIX.

After the MS. had gone to press natural infections of the virus were found out-of-doors upon a number of *Datura* plants which were being grown for experimental purposes in the vicinity of Cambridge. These plants were growing near potatoes and were surrounded by fields of corn. They were about half a mile from the nearest tomatoes and from the experimental glasshouses. This seems to indicate that the virus is insect borne and that it is harboured by some wild host plant in the hedgerows from which it may have come in the first place to tomatoes.

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OBSERVATIONS ON THE SHEEP BLOWFLY (*LUCILIA SERICATA* MEIG.) IN SCOTLAND

By F. N. RATCLIFFE.

(*Marischal College, Aberdeen.*)

(With 2 Text-figures.)

RESEARCH into problems connected with sheep blowflies (or maggot-flies, as they are more usually called in this country) is at present being carried out in at least three centres in Britain. To some extent the work at Aberdeen has overlapped that undertaken in North Wales; but, in view of the nature of the species under consideration, it seemed advisable to publish, in an abbreviated form, the results obtained in Scotland. *Lucilia sericata* Meig., the species which is almost exclusively responsible for maggot trouble in Britain, is a pest of sheep in many countries of the world, *e.g.* Europe, North America, South Africa, and Australia. While there is no evidence that definite physiological races have been evolved, it would on the whole be surprising if examples from such widely differing environments showed complete uniformity in their life history and behaviour. The results already obtained by different workers show slight discrepancies, which may be significant and point to real physiological differences between the local populations.

CALLIPHORA ERYTHROCEPHALA MEIG. AS A SHEEP MAGGOT-FLY.

As in North Wales (Davies, 1934) it was found that the bluebottle, *Calliphora erythrocephala*, can hardly be regarded as a sheep-striking species. It was bred (together with *Lucilia*) from only one sample of maggots, obtained on upper Donside in Aberdeenshire, from sheep grazing at 900 ft. altitude. There is some slight doubt as to whether even this can be considered a genuine record, as the time taken by the larvae to reach their full growth pointed to the possibility of an infection of the meat in the laboratory (though it is hard to see how this could have escaped notice).

During the breeding experiments it was found that a temperature of 90° F. (32° C.) was fatal to the pupae of *Calliphora erythrocephala*, which indicated that this species was adapted to a lower temperature range than *Lucilia sericata*. Experiments at present in progress at Aberdeen (the results of which will be published in due course) have shown that the larvae also cannot survive this temperature, or even 4–5° lower. The practical significance of this is obvious. It means that maggots of

Calliphora erythrocephala cannot develop in contact with the skin of a living sheep, especially in those parts (the breech and under the legs) where moisture conditions are usually most suitable, but where the temperature approximates to blood heat (102° F.).

Consideration of the temperature factor lends theoretical support to Davies' suggestion that *Calliphora erythrocephala* behaves in this country as a "secondary" fly, *i.e.* only striking sheep on which larvae of *Lucilia* are already feeding (Davies, 1934). *Calliphora* will only find suitable temperature conditions in the fleece some distance from the skin; but it is not until a strike has proceeded for a certain length of time that nourishment, in the shape of the serous ooze which the feeding maggots provoke, will be available there.

Scottish shepherds firmly believe that the early season strikes (*i.e.* in June) are due to the bluebottle, the green *Lucilia* only appearing later in the season. It is possible that the belief has a sound observational basis; for *Calliphora* is undoubtedly attracted by the scouring ewes which are the chief victims in the early part of the season (see strike records below).

BREEDING EXPERIMENTS WITH *LUCILIA SERICATA*.

For mating and oviposition a large cage, 37 by 22 by 20 in., was used. The top was of glass, the sides of a transparent, cellophane-like substance. This cage was kept in front of a window which received the morning sun. To provide extra light and warmth a fairly powerful electric bulb was placed inside the cage (the light usually being turned off at night). While this was burning a fairly constant temperature of 20°C. (70°F.) was maintained in the cage. A beaker of water, in which a sponge was half-submerged, kept the atmosphere moist. Fresh steak mince was provided for food (and for oviposition), also some syrup in a watch-glass.

Larvae were fed in beakers (also on steak mince) covered with stout muslin. When the larvae were fully fed the beakers were placed in the tops of glass jam jars, quarter- to half-filled with powdered cork packing; and the whole covered with muslin. This arrangement allowed the larvae to leave the food when they desired. Cork packing was selected because it was clean; and, being lighter than sand or earth, less liable to damage larvae or pupae when batches were tipped out for examination. Pupae, when collected and segregated, were placed in small muslin-covered jars without cork. The larvae and pupae were kept in the dark; and care was taken to keep the cork packing, and the muslin covers of the pupa jars, moistened.

Three temperature environments were used in the experiments: 32°C. (90°F.), 27°C. (80°F.), and room temperature. A record of the last was kept (maximum and minimum); and during the months of August and September, when the larvae were feeding and most of the pupation records were taken, it varied as follows: maximum from 59 to 72°F., minimum from 55 to 63°F. Actually the temperatures were more constant than these figures indicate.

Oviposition.

Three females and three males were put into the cage on August 26th and 27th, 1933. On August 29th one female and two males were dead. Two females and three males, making *four pairs*, were then put in the cage. One male subsequently died and was replaced.

Mating was first observed on September 3rd. The female had a peculiar, slow, droning flight, which seemed to excite the males.

Egg batches when discovered on the meat were removed and placed in corked vials. The newly-hatched larvae were counted by washing them on to dark-coloured blotting paper, which showed them up readily. The following egg batches were laid.

Date						No. of eggs in batch
Sept. 3	(Plus two infertile batches)					190
" 4	269
" 5	220
						215
						48
						254
" 6	229
	(Plus three uncounted batches)					
" 7	220
						172
						233
" 10	202
						258
						175
" 11	159 plus
" 12	Three batches, total					230 (approx.)
" 14	690
" 15	262
						117
						253
						210
" 16	173 (first female died)
						44
						203
" 17	246
" 18	Three batches, total					624
" 20	190 (second female died)
						227
" 22	232
" 25	198

(The last two females died on September 29th and October 1st. One, which was drowned in the syrup, had wings so worn that it could hardly fly. The ovaries were packed with eggs, seemingly ready for oviposition.)

This gives a total of 6743 eggs, plus an unknown number in the three uncounted batches of September 6th and the two infertile batches. They were laid in thirty-three batches in the 22 days from September 3rd to September 25th: the equivalent of 1685+ eggs per female. The first batches were laid 7-8 days after the flies (newly emerged) were put into the cage. Allowing for the uncounted and infertile batches, the average number of batches laid by each female was nine, which gives an average interval between ovipositions of between 2 and 3 days, being the period necessary for the maturation of a fresh supply of eggs in the ovary.

The combination of favourable conditions of this experiment, with a constant warm temperature, an abundance of protein and carbohydrate food, and individuals bred from fully fed larvae, could rarely be reproduced in the field. The results in consequence probably exaggerate the egg-laying power of the flies in nature, especially as regards the size of the egg batches laid. In examining batches found on living sheep it was noticed that the smaller clumps usually contained between three and four dozen eggs. The largest clumps were obviously the result of several females laying together, and in some cases contained over a thousand eggs. It is impossible to say, of course, whether forty to fifty eggs represents the normal number laid under natural conditions. It seems likely, however, that this is a closer approximation to the figure than the 250 obtained in the laboratory experiments.

The laying of one of these large egg batches was found to occupy 30 min.; and was preceded by more than half an hour's careful exploration of the meat. Examination of "blown" sheep shows that the flies, to reach a spot considered suitable for oviposition, will often force their way to within half an inch of the skin, and in the middle of a staple.

Feeding period of larvae.

The length of feeding time, as judged by the observation of large numbers of larvae, was as follows (the starting-point being unfed, freshly hatched larvae, the end being the voluntary wandering from the food vessel):

Room temperature	6-9 days
80° F. (27° C.)	3-4 "
90° F. (32° C.)	2-3 "

Prepupal stage and hibernation.

When fully fed the larvæ leave their food and enter into a definite stage of wandering, followed by dormancy. Their appearance remains more or less unchanged, except that the food in the forepart of the gut disappears, and the imaginal discs attain a pale salmon colour which shows clearly through the skin. The experiments threw very little light on the factors controlling the length of the dormant stage, or of the probable length of this stage under natural conditions. The environment of the larvae in the laboratory was highly unnatural, involving a daily disturbance for examination. The individual variation in the length of the prepupal period was so great that it was clearly unsafe to draw deductions from a comparison of different batches, even when they contained between 50 and 100 larvae.

It was found that at room temperature the dormant period tended to be so prolonged that an insignificant number of pupations were recorded among the hundreds of larvae bred. This reluctance to pupate is exemplified by the history of a batch of some 160 larvae which were fed at 80°F., and transferred to room temperature when they had wandered from their food. Seven individuals pupated within the first week (some of which were probably influenced by the high feeding temperature, for they may have wandered a day or two before the others). In the three months before the batch was removed to an unheated environment only seventeen further pupations were recorded.

At the incubator temperatures the dormant period was much shorter, though the variation was so great that in one or two cases pupation was postponed for more than 70 days. There seemed to be no significant difference between the batches at 80 and 90°F. Normally there was a rush of pupations in the first few (5–10) days after feeding stopped, after which odd larvae would pupate over a period of a month or more. In these batches the percentage of larvae which had a dormant period of *a week or less* averaged about fifty, this figure being fairly consistent. One or two batches did not show this early rush and appeared to be exceptional.

Larvae which had been dormant for some time at room temperature could be induced to pupate rapidly if placed in one of the incubators. The effect of a sudden rise in temperature seemed to increase with the length of the dormant interval. Thus a batch of forty-one larvae were placed in the 80°F. incubator after being dormant for 14 days at room temperature: in 1 week 80 per cent. had pupated. A batch of 100 were

placed in the incubator after a dormant period of 1 month: 63 per cent. had pupated in a week. Of two large batches (both of over 100 larvae) which had been dormant for 2 months and 19 days at room temperature, one achieved 100 per cent. pupation (except for one individual) in 2 days, the other (except for two or three) in 3 days.

It is now well established (Davies, 1934) that *Lucilia sericata* normally hibernates in the dormant larval stage. In order to see whether they could survive the winter as pupae, the two large batches mentioned in the preceding paragraph were taken, immediately after their removal from the incubator on November 11th, to an unheated cellar. Here the temperature varied from a maximum of 50° F. to a minimum of 34° F. All the pupae were dead when examined in April; many, however, contained fully formed flies. (These pupae were kept in jars half-filled with cork packing, which it was found unnecessary to moisten as the cellar was sufficiently humid to prevent desiccation.)

Dormant larvae survived this period under similar conditions. On April 28th they were removed to an unheated bedroom. On May 9th there were no pupations. On May 14th pupation had started; and by June 27th all but three individuals had pupated. The temperature readings during the critical period were as follows:

	Maximum °F.	Minimum °F.
Month of April (in cellar)	49	45
May 1	54	44
" 9	57	48
" 14	62	43
" 20	55	42
" 28	60	46

Lacking knowledge of the other factors concerned, it is unsafe to deduce much from these figures. They indicate, however, that a temperature of 55° F. (12.5° C.) is necessary to induce pupation.

Pupal period.

Experiments showed that the length of the pupal period was fairly constant for each of the three temperatures used, although a far greater variation was shown here than in the feeding period of the larvae.

At room temperature the time varied round 3 weeks. In no instance did it exceed 24 days, while one outstanding record of 11 days was noted. The following is the analysis of the batch which showed the widest variation:

Days ...	11	16	17	18	19	20	21	22	23	24
Number ...	1	2	8	9	15	21	16	21	11	5

At 80°F. (27°C.) the period was 6 or 7 days. Two batches, in which the larvae were also fed at 80°F., worked out as follows:

		(a)		(b)			
Days...	...	6	7	5	6	7	8
Number	...	45	22	9	45	33	3

At 90°F. (32°C.) the time was slightly shorter. The sum total of all these batches (larvae also fed at 90°F.) worked out as follows:

Days	...	4	5	6	7
Number	...	3	96	69	3

Average 5.4 days.

The above results are not directly comparable with those of Davies who employed conditions more closely approximating to those of nature. The pupal periods at 80 and 90°F., for instance, have little bearing on British conditions where such temperatures would never be attained in the soil. It is interesting, however, to compare these figures with those of Cousin (quoted by Davies), who worked out the life history of *Lucilia sericata* under constant controlled conditions, at a temperature of 33°C. (91.4°F.). Her average feeding period for the larvae was 3 days; mine, for 32°C. (90°F.), was between 2 and 3 days. Her pupal period was 8 days, mine 5.4 days. This difference seems large enough to be significant, and may possibly indicate a physiological difference between the French and Scottish "races" of *Lucilia sericata*, the latter going through their life history rather more rapidly at a given temperature, as might be expected in a more northern "race".

Breeding experiments during 1934 broke down owing to the failure to induce mating. This may have been due to the fact that a larger number of rather smaller cages were employed. Attempts, carried out over a number of weeks, to produce artificial strike by placing batches of eggs on penned lambs (kindly provided by the Rowett Institute) also failed. This failure is readily explained by the findings of Davies and Hobson (1935), who, working with Welsh sheep, discovered that the humidity in the fleece, even next the skin, rarely reached the figure necessary for the successful development of the eggs, except in the region of the breech.

Field observations.

The results of field observations, especially those directly concerned with the practical aspect of the sheep blowfly problem, have been published in the *Scottish Journal of Agriculture* for July 1934. They emphasised the extreme importance of the factor of the attractiveness of the

sheep to the flies, for trapping experiments (the results of which it is hoped to publish in the near future) showed that only a very small proportion of the flies in the neighbourhood of the flocks actually struck sheep.

During the 1933 and 1934 seasons the shepherd of the Rowett Institute flocks kept day-to-day records¹ of the animals struck, which are here reproduced in the form of graphs (Figs. 1 and 2). The flocks were grazed in a number of fields at the base and round the lower slopes (north-west, north and east) of a hill some 900 ft. high. The altitude of

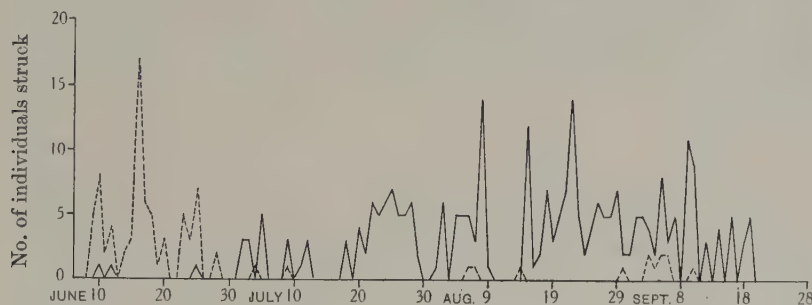


Fig. 1. Graph showing incidence of "strike" in a flock in 1933. (Broken line refers to ewes; continuous line refers to lambs.)

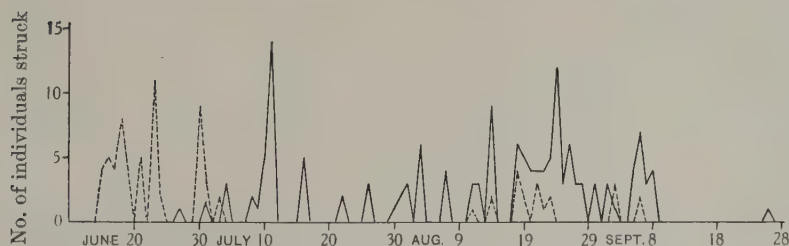


Fig. 2. Graph showing incidence of "strike" in same flock in 1934. (Broken line refers to ewes; continuous line refers to lambs.)

the pastures is between 400 and 600 ft. above sea-level. They can be described as fairly open to moderately sheltered.

Although the field in which each animal was struck is noted in the records, it is not easy to deduce from them much as to the environmental

¹ These records, unfortunately, cannot be considered quite complete. On his own confession the shepherd failed to note several cases; and it seems most likely that these omissions would occur at the busiest times, *i.e.* when he had a large number of animals to treat (thus the high points on the graph should probably be still higher), and when the dipping and clipping were in progress. Actually, therefore, the percentage figures obtained from these records underestimate the seriousness of the position.

factors (shelter, etc.) affecting the incidence of strike. The sheep were divided into small flocks, largely according to type and breed, which grazed the fields more or less in rotation. It is impossible to tell from the records how many days each group of animals was kept in a certain field, or how many individuals comprised the group. Comparison between fields is, of course, meaningless if they were being grazed at different times; thus nothing can be gained by comparing the total strikes for each field over the year.

The following is an example of the results obtained by analyzing the records of the different fields. From June 9th to July 5th, 1933, six of the fields carrying sheep (during part or all of this period) gave these strike totals:

Field No. 40 (7.5 acres). One of a group of level fields, stone dyked and relatively unsheltered—23 cases.

Field No. 36 (12.2 acres). Adjacent to 40; nearer the slope of the hill; includes a sheltered hollow—27 cases.

Field No. 38 (15.7 acres). Separated from 36 by a road only; on foot of hill slope; one corner sheltered by farm buildings and windbreak of beech trees—7 cases.

Field No. 39 (16.7 acres). Adjacent to 38; farther up hill slope; quite open—10 cases.

Field No. 47 (4 acres). Probably the most sheltered field of the pasture; wood on two sides—8 cases.

Field No. 48 (5.9 acres). Adjacent to 47; not quite so sheltered—7 cases.

As far as the first four fields are concerned, it may be said that the incidence of strike agrees fairly well with expectations based on the assumption that shelter from wind (so long as it does not introduce too much shade) encourages the flies. Anticipating the publication of the trapping experiments (carried out by Dr Guy Morison), it may be mentioned here that a trap set under the wire fence separating fields Nos. 36 and 40 proved the second most successful of the series, being surpassed only by the trap set farthest up the hillside.

The small number of cases recorded in fields Nos. 47 and 48 can probably be explained by their small size, and the consequent fact that they were grazed for shorter periods.

Taking the records of the total strike numbers over the season, as shown in the graphs, the main features of the seasonal course of events are immediately apparent. The early strikes, in the month of June, are

confined almost entirely to ewes, mostly to scouring ewes, and the site chosen for attack is the breech and root of the tail. The lambs at this age have not sufficient fleece to attract the fly (the case recorded on June 12th, 1933, of a lamb struck on the back, is quite exceptional). When the ewes are clipped at the end of June they become practically immune until towards the end of the season, when they begin to be struck again, but in much smaller numbers than the lambs. These late cases, both lambs and ewes, are mostly back and body strikes; though a scouring animal will be struck near the tail.

The lambs are summer dipped just after the ewe clipping. This, added to the fact that their fleece condition is not yet fully "ripe" for strike, tends to make the beginning of July a relatively free period. (The late dipping, of the *whole* flock, was carried out in the last week of August in 1933, and on August 9th in 1934.)

When these complicating factors are taken into account (*viz.* the clipping, dipping, gradual development of the fleece of the lambs to a condition which will encourage strike, and in addition the variation through the season of the population of the flies themselves), it is only to be expected that very little correlation between the graph of strike incidence and meteorological conditions will be apparent; although the weather is undoubtedly a factor of paramount importance in the matter. The meteorological records for periods of the 1934 season which showed significant differences in strike incidence (*e.g.* July 12th–25th and August 18th–28th) were examined; but the results were such that it seemed profitless to pursue this aspect of the matter further. It may be mentioned, however, that the records did provide a satisfactory explanation of the high strike incidence on July 10th and 11th, 1934. The 8th and 9th (the dates on which the actual oviposition would have taken place) were exceptionally hot and humid, with fog at night over the hill; and on July 10th there was a drop in the amount of sunshine, the humidity keeping high.

Comparing the years 1933 and 1934, it may be said that in the opinion and experience of the shepherd the former was quite exceptional, and the latter worse than normal. As the size of the flock altered month by month owing to sales and purchases (see Table I) it is not easy to arrive at a figure which will represent the strikes as a percentage of the total flock. However, if the total flock is taken as the average of the four monthly tallies, giving 1062 for 1933 and 857 for 1934, the number of cases of strike over the whole season (362 and 227 respectively) work out at 34 per cent. for 1933 and 27 per cent. for 1934.

Table I.

Showing the number of strikes recorded during each month throughout the 1933 and 1934 seasons, and the variation in the size of the flock. (Figures supplied by the Rowett Research Institute.)

	Number of cases of strike		Tally at end of month		
	Ewes	Lambs	Ewes	Lambs	Total
1933					
June	72	3	463	734	1197
July	2	69	462	644	1106
Aug.	4	129	462	553	1015
Sept.	8	75	498	433	931
Total	86	276			
1934					
June	52	1	472	592	1064
July	5	40	464	523	987
Aug.	15	86	485	388	873
Sept.	5	23	334	169	503
Total	77	150			

Making a similar calculation for the two classes of animals, ewes and lambs, it is found that the difference between the two years is due entirely to a difference in the figures for the *lambs*. The percentage strike in the ewes is the same, 18 per cent. to the nearest integer, for both 1933 and 1934. For the lamb flock it is 47 and 36 per cent. respectively.

These figures agree well with those of Davies for North Wales, who states that shepherds in exposed lowland districts consider a 20–25 per cent. incidence severe, and in wooded lowland districts 35–40 per cent.

Only one other point in connection with these strike records needs mentioning. During 1934 the shepherd noted which of the cases were “restrikes”, *i.e.* the result of oviposition round an existing strike patch. His figure of 16 (though owing to the nature of his recording this must be taken as a slight underestimate) seems very low; but this again agrees with Davies’ findings. The number of restrikes depends very largely on the care taken in treatment; and where the effect of the dressing used on the sheep’s skin is ignored, the problem of restrikes may be very serious.

SUMMARY.

1. It is suggested that a limited temperature tolerance in the early stages of *Calliphora erythrocephala*, as compared with *Lucilia sericata*, is the reason for this species not being a serious pest of sheep.

2. Experiments to discover the numbers of eggs laid by females of *Lucilia sericata*, and the length of the larval, prepupal, and pupal stages are described.

3. The detailed records of strikes in an Aberdeenshire flock throughout the 1933 and 1934 seasons are analysed; and the figures shown to agree with results obtained in other parts of Britain.

NOTE. These observations form part of the results of an investigation of the sheep blowfly problem in Scotland, carried out under the general supervision of Professor James Ritchie by aid of a grant from the Agricultural Research Council.

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THE ALIEN ELEMENT IN THE BRITISH SAWFLY FAUNA

By ROBERT B. BENSON, M.A.

(*Department of Entomology, British Museum (Natural History).*)

INTRODUCTION.

THE alien sawflies of Britain are of especial interest to the economic biologist because many of the species that have given trouble in agriculture, horticulture or silviculture appear to have been aliens in our fauna. They were apparently introduced into the country together with the plant they attack. This alien element represents about $7\frac{1}{2}$ per cent. of our total sawfly fauna; that is thirty out of about 400 known in Britain. Some of the species, apparently well established, or at times even pests in our gardens, are unrecorded as British except in journals not easily accessible to the economic biologist. As no one before has attempted to give a summary of our aliens, I venture to do so now. Such a summary is the work for one whose interest is primarily in systematics.

Systematics is not a pure science; it can perhaps be described as applied morphology. Nevertheless, owing to the immense number of species of animals in the world and the extreme difficulty of separating them from each other for identification, systematics has come to be the basis of all other biological science, for only with the correct name of an animal as a key is it possible to unlock the literature and discover what is already known about it.

The extreme difficulty of systematics is perhaps not fully realised by those not primarily interested in the subject. The systematist is generally expected to give a final verdict on totally inadequate evidence. He is often expected to form a comprehensive idea of a species, that is a population of a great many individuals running into, possibly, hundreds of thousands, no two of which are absolutely alike, when he is given to examine only two or three individuals that have met with rather a violent death and show, quite naturally, individual differences; moreover, he has perhaps never seen any insect at all like them before. He is expected to know, from a cursory examination, the limits of variation within this species of size, colour, proportions of all the parts, sculpture,

wing-venation, etc., and how to distinguish it from all other closely allied species, even though he may never have seen them.

The lack of good long series of specimens generally prevents the systematist from ever learning the range of variation of a species. Speaking as a student of the Hymenoptera, I consider that our museums badly need long series, running into hundreds, of well-preserved individuals of even the commonest species. Such material could be supplied at times by economic biologists when they are breeding species in large quantities. Much greater co-operation between biologists and systematists is desirable, for one of the greatest difficulties the systematist has to face is his ignorance of the living animal and his lack of opportunity to get this knowledge. Problem after problem in systematics will finally have to be solved by breeding experiment, genetical, cytological or biochemical study.

Another great difficulty in systematics lies in the definition of the term species. The old concept of the fixed Linnean species was formed before the evolutionary idea had permeated biological thought. Our present concept must include this idea of evolution and several other ideas; that it is possible, for example, for individuals of a species to overlap morphologically with those of another species, that it is possible to have intermediates between species, and that within a species there may be distinct forms remaining isolated and without intermediates though they are interbred.

Nomenclature has its own difficulties to contribute, but I will not touch on them here.

Unfortunately much work on ecology, distribution and biology is almost worthless through inadequate systematics, for the importance of many of these studies depends on a final and accurate determination of species. Now, because systematics grows and develops like any other study, it follows that every specimen whose identity is important, every specimen on whose identity is based some biological, ecological, or other information, *must be preserved*, so that it may be re-examined every time any new conception of the systematics of that group is made. In this way only can any knowledge of these subjects be built upon a sound basis. Unless this is done the records become quickly out of date and worse than useless because they mislead. In trying, for example, to collect information on the distribution of sawflies in Britain I have found that I must ignore nearly all the county lists published heretofore, unless I am able to check the determinations or unless Dr R. C. L. Perkins, F.R.S., has already done so; this does not apply only to the more critical

groups but even to the most obvious and striking species. A good example of this concerns the occurrence of *Orussus abietinus* Scopoli in Britain. Morice (1904, p. 35) stated that on the present evidence the species could not be regarded as British; and a month later, on p. 49, he corrects himself as a result of a letter he had received from a correspondent, who recorded a specimen taken 20 years before near Hastings. Then, by chance, a specimen bearing the recorded data and identified as "*Oryssus abietinus*" recently came into the hands of H. M. Hallet of the National Museum of Wales, who informs me that the specimen is not an Orussid at all, but a perfectly normal specimen of *Xiphydria prolongata* Geoffroy. Had this specimen not been preserved, or had it got lost, we should never have known the truth of this record.

Owing to the frequency with which introduced insects become pests for a time, it is important to ascertain what species seem to have been introduced recently into Britain, and to keep a careful watch for new ones that are establishing themselves. The process of converting an alien into a normal member of the fauna can be helped by the introduction of its natural enemies.

The normal process seems to be that shortly after being introduced into a new country, a species which succeeds in establishing itself may multiply for a time exceedingly. After a while it tends to decrease in numbers and to become a normal member of the fauna, or perhaps to disappear altogether. The reason for this is often perhaps that the species is in the first place introduced without its natural parasites, or under conditions unsuitable for the parasites to survive, and that its other enemies have not learnt to attack it, but that, after a while, the insect or other parasites of some closely allied native species, or some larger predaceous animal, turn their attention to the introduced species, until a natural balance or extermination results. These parasites, or other enemies, may even be races of the same species that attack the alien in its native home.

In the following discussion, the arrangement adopted is primarily based on plant classification, starting with the Dicotyledones with the various plant orders alphabetically. Under each order, either all species of sawflies attacking the order are taken directly alphabetically, or else the order is subdivided first into plant genera alphabetically. The plant names throughout are according to the system of nomenclature used in the tenth edition of Babington's *Manual of British Botany*, edited by A. J. Wilmott. I am also indebted to Mr A. J. Wilmott personally for further information on some of the plants discussed, and for helpful criticism.

For what is known on the origin of our cultivated plants I have made great use of the little book by Alphonse de Candolle (1884), which still remains the standard book on the subject, although more recent additions to our knowledge have in certain genera made it necessary to modify some of his conclusions; and, on our trees, I have consulted Elwes and Henry's *The Trees of Great Britain*.

With each sawfly dealt with a reference is given to a published description of the larva and biology. Wherever possible the references selected are from Cameron's *Monograph of the British Phytophagous Hymenoptera*, but when the information is not in this book, then Enslin's monograph *Die Tenthredinoidea Mitteleuropas* is selected. In certain instances other more recent references are given instead.

Some of my indebtedness to Dr R. C. L. Perkins, F.R.S., is obvious from the many references to his work in the account that follows; I am also indebted to him for much personal help and encouragement, and for the very idea of writing this paper.

DICOTYLEDONES.

CRUCIFERAE.

A number of plants belonging to this order are cultivated as vegetables. Two sawflies were known on these in Britain in the past:

- (1) *Athalia rosae* Linné auct. (= *colibri* Christ. and *spinarum* Christ.).

This species was well known to the earlier writers as a serious pest on turnips (*Brassica campestris* Linné), and it also attacked other Cruciferous plants such as radish (*Rhaphanus sativus* Linné) but not cabbage (*Brassica oleracea* Linné). Newport (1838), in his remarkable account of the anatomy and habits of the insect, says that its favourite food is charlock (*Sinapis arvensis* Linné), and that it will attack this in preference to turnips growing in the same field.

A description of the larva and summary of its life history can be found in Cameron (I, 308-12), and he gives *Barbarea* and *Sisymbrium* also as food plants. The first records of this species in Britain were published by Marshall (1783). He said that the larvae had been particularly harmful about 1760 and again in 1782. Yarrell (1837) mentions 1818 as a year when it again occurred in abundance, and then from 1833 onwards it occurred annually, though waxing and waning with the seasons, until shortly after the accession of Queen Victoria and the general introduction of the rotation of crops when it slowly declined again. As a

member of our fauna it has now been practically extinct for the last 30 years (see Benson, 1931).

Marshall (1783) suggested that the species had come to this country as an immigrant about the middle of the eighteenth century, and notes that it was first observed on the east coast, where it appeared in clouds. From the east coast it spread over the British Isles, but according to Cameron it was probably never a serious pest in the north of England or in Scotland.

Yarrell (1837) speaks of the species as thriving in those seasons only in which there had been an almost total absence of rain, and suggests that while the hot dry summers favoured the spread of the species, the cold wet ones checked it. The species does still occur occasionally in Britain. I have four records for the last 30 years; each of these is from the sea coast which suggests that the specimens referred to were also immigrants. Thus Britain appears to be outside the normal range of this species. Probably it cannot normally survive our winter climate, and its presence here was probably occasioned by large swarms of immigrants in the spring which multiplied rapidly in a hot dry summer.

It is not now apparent what factors led first to the increase and spread of this species in Britain and then to its gradual decline and almost complete disappearance. The introduction of crop rotation has been suggested as the cause of the disappearance of the species, but it is difficult to see how the mere changing about of the fields under turnips from one season to another could have had this effect. A species that could find its way across the channel to colonise new lands would surely not find a hedge or a field or two on a farm an insurmountable barrier between it and its food plant.

This species is a pest throughout central and southern Europe and Asia as far east as Japan. *Athalia lugens* Klug, which in India and Japan is also a pest on the same plants as *A. rosae* Linné, occurs locally in Britain but not on cultivated plants and has never been recorded as a pest. Certain other of our native *Athalia*, such as *A. liberta* Klug, *A. cordata* Le Peletier and *A. lineolata* Le Peletier, also occur on Cruciferous and other plants in gardens.

(2) *Tenthredo flaveola* Gmelin (= *flavipes* Geoffroy).

Curtis (1839) records that Mr Shuckard had found this conspicuous species in abundance in Battersea fields at the end of June. Wanting to see the species there himself, he went at the beginning of July and found two females and also larvae on *Sinapis nigra* Linné and *S. alba* Linné, but these he failed to rear.

F. Smith also went to see it and obtained larvae which he succeeded in rearing. Shortly after this the species disappeared, whether before or at the time of the draining of Battersea fields is not known. Since that occasion the species has hardly ever been found in Britain. Morice (1912, p. 158) records only two other British specimens known to him, a male from the Oxford district and a female from Colchester (Harwood). I know of no other records. There is a beautiful coloured figure of the larva in Curtis (1839), and this is copied with the description in Cameron (I, 147, and Plate I, fig. 8) as *Allantus flavipes* Geoffroy. The sudden swarming of the species in Battersea fields and then its disappearance suggest that the species was an alien.

LEGUMINOSAE.

Robinia Pseud-acacia Linné.

Pteromidea tibialis (Newman) = (*P. trilineata* Norton).

This species, which was introduced into Britain from North America with its food plant, was described first from introduced British material by Newman (1837), 30 years before it was discovered in its native country when it was again described as new by Norton (1867). The species is generally to be found in Britain wherever *Robinia Pseud-acacia* Linné is cultivated, that is, mostly in the southern counties. According to Elwes and Henry (VI, 1502), the exact date of the first introduction of this tree into Britain is uncertain, but it was probably near the beginning of the seventeenth century. However, it was not commonly planted and "it only came prominently into vogue as a result of the vigorous advocacy of Cobbett who began to write about 1823...". A detailed description of the larva can be found in Cameron (II, 131) as "*Nematus tibialis* Newman".

ROSACEAE.

Aruncus silvester (Kosl.).

Pteronidea spiraeae Zadd.

This species is sometimes very destructive to *Aruncus silvester* (Kosl.) in gardens round London and probably elsewhere. A detailed description of the larva and adult, together with a summary of all that is known about its life history and distribution, is given by Robbins (1927). The shining yellowish green larva with a darker stripe down its back feeds gregariously on the undersides of the leaves, devouring irregularly rounded holes between the veins, and sometimes entirely defoliating the plants.

In the colonies that I have observed the species appeared to be several brooded, the number of broods normally at least three, depending on the length of the season, but in midsummer the broods overlap to some extent so that in July eggs, larvae in various stages, as well as adult flies can be found at the same time. The species was first noticed in England in 1924, when it was discovered in Hertfordshire, but the plant has been in cultivation in Britain since 1633. Although the sawfly is only known wild in Bavaria, the food plant has a wide holarctic distribution, occurring commonly in woods in many parts of Europe, Asia, and North America, though it is not native in Britain.

Geum.

Metallus gei (Brischke) (= *Entodecta gei* Brischke auct.).

This species is established at any rate in the south of England, where it can be found in gardens, particularly near or in London, mining in the leaves of various forms of *Geum*. Sometimes up to eight larvae can be found in a single leaf, the mines becoming fused, as the larvae grow, until the whole leaf is eaten out by a single large mine.

The species can be found on wild *Geum*, as well as cultivated varieties, and it is not possible to say whether or not the species is a true native. In my experience it is much more abundant in gardens and occurs wild mostly only in the immediate neighbourhood of gardens. Recently, however, I found it mining *Geum urbanum* Linné in woods in Buckinghamshire, on Duncombe Terrace, near Ivinghoe, where it appeared to be wild, as it was more than a mile from the nearest cultivated garden. Enslin (p. 301) says that there is apparently only one brood a year in Germany. In Hertfordshire there are certainly two broods, as I find the mines not only in June and early July, but also again late in the autumn, in September, October and November. The species has only once been recorded as British. This record was based on a single specimen by Dr R. C. L. Perkins, F.R.S. (p. 296), who tells me he has since obtained the species in greater plenty in Devonshire and that he has also seen specimens from near Oxford. I have found the species myself in gardens at Boxmoor, Hertfordshire, and also, as mentioned above, near Ivinghoe, Buckinghamshire, and in the grounds of the British Museum (Natural History). Dr O. W. Richards has shown me specimens of the larvae obtained from *Geum* in the grounds of the Entomological Field Station of the Imperial College of Science at Slough, Buckinghamshire. In addition to this the late Mr J. C. Robbins found larvae mining *Geum rivale* Linné at Limpsfield, Surrey, in July 1931; some of these larvae were successfully

bred and I have seen the specimens. Mr J. A. Simes, O.B.E., tells me that he has been bothered with it for two or three years now on cultivated *Geum* in his garden at Loughton, Essex.

Abroad the species is known only in France, Germany and eastwards through Russia to the Caucasus.

***Pyrus communis* Linné.**

Micronematus abbreviatus Hartig.

This species has been mostly overlooked in Britain. Cameron (II, 61) mentions only a single specimen taken at Braemar by Dr Sharp and this, now in the British Museum, was certainly wrongly determined as it belongs to a species of *Lygaeonematus*; the description of the larva in Cameron does, however, belong to the species in question as it was copied from van Vollenhoven. Up till now the species has only on one other occasion been recorded as British: Dr R. C. L. Perkins, F.R.S. (pp. 206-7), found the larvae on pear leaves growing in a garden in Newton Abbot, Devonshire. He bred specimens for some years and found that the species is only single brooded, but he never obtained any males, nor would the species feed on apple as stated by van Vollenhoven.

In the Morice collection at the Hope Department, Oxford, there is another specimen taken by Morice at Woking, Surrey. In July 1930, I found larvae feeding singly on pear against a wall in a garden in Berkhamsted, Hertfordshire, and my colleague, Mr G. E. J. Nixon, has brought me a larva which he found in similar circumstances in his garden at Tulse Hill, London, S.E.

The species is recorded as harmful in Central Europe but does not seem to be common enough in Britain to do any serious damage, although it is probably more widely distributed than is generally realised. The fact that all the records of its occurrence in Britain, so far, are from sheltered gardens suggests that it may not be a true native, and according to Elwes and Henry (VI, 1562) the common form of the pear tree is only a doubtful native of Britain, although *Pyrus communis* var. *briggsii* Syme seems to be an endemic confined to south-west England.

A closely allied sawfly, *Micronematus monogyniae* Hartig, feeds on the leaves of wild sloe (*Prunus spinosa* Linné) and is not uncommon in the south of England in very early spring, but it has not been recorded on cultivated *Prunus*.

***Pyrus malus* Linné.**

The well-known apple-boring pest *Hoplocampa testudinea* Klug is probably a native of this country. The cultivated apple is derived, in part at least, from our native wild stock, and I regularly find *H. testudinea* Klug infesting crab apples in Hertfordshire. *Lygaeonematus maestus* Zaddach which feeds on apple leaves I have also found on crab apple and it may perhaps be a native.

Hoplocampa flava* Linné.**Prunus.***

Generally *Hoplocampa flava* Linné must be looked for on cultivated plums, while *H. chrysorrhea* Klug and *H. rutilicornis* Klug, which also occur in Britain, must be looked for on our wild sloe. Dr H. W. Miles has sent me for examination a few specimens of *H. chrysorrhea* Klug collected from the blossom of cultivated plum in Lancashire, Cheshire and Cambridgeshire; these are the only records I know of this species ever having been found associated with cultivated plums, and these were possibly no more than chance visitors to the blossom. According to Enslin (p. 246), *H. flava* Linné is found on various species of *Prunus*, but specially favours *P. avium* Linné, *cerasus* Linné, and *spinosa* Linné: this is not in accordance with our experience in England, where we have never yet met with it on *P. avium* Linné or *cerasus* Linné, and not commonly on *P. spinosa* Linné. Our form appears to be a biological race on cultivated forms of *P. domestica* Linné.

Our cultivated plums, damsons, etc., appear to be derived from more than one wild species of *Prunus*; our native *P. insititia* Linné is probably one of these. *P. domestica* Linné is not, however, a native of Britain or North Europe, and according to de Candolle (p. 214) occurs naturally in Anatolia, the region to the south of the Caucasus and northern Persia. This is, then, suggested as the original host of *Hoplocampa flava* Linné, which has been introduced into northern Europe with its host plant. Two recent papers on the biology of this species have been published: Petherbridge, Thomas and Hey (1933), and Miles, Thomas and Hey (1933). *H. minuta* Christ. was at one time also recorded in this country, but, as I have already pointed out, I have never seen any British specimens of this species (see Petherbridge, Thomas and Hey); it is said by Enslin (p. 251) to feed only on *Prunus domestica* Linné and *P. Armeniaca* Linné, the apricot, a native of China. As the known distribution now of the sawfly is throughout most of Europe, together with Bessarabia and Turkestan (Sprengel), it is suggested that the original host of this species also is one of the forms that go to make *P. domestica* Linné.

CONIFERAE.

By far the greatest number of introduced species are found on plants of this order. To take first the Siricidae: *Urocerus gigas* Linné, *Sirex noctilio* Fabricius, and *S. cyaneus* Linné are at the present day well established in Britain, attacking *Larix*, *Picea* and *Pinus*. *Sirex juvencus* Linné is more doubtful. The present position of these species in Britain and an account of their biology is given in a paper by Chrystal (1928), and a summary of their status in Scotland is given by Evans (1922). *Urocerus gigas* Linné and *Sirex noctilio* Fabricius are possibly native to the ancient pine forests of Scotland, etc., although the modern transport of timber has introduced many specimens of these species in the larval form into the country, together with representatives of other species. There is some reason to believe that *S. noctilio* Fabricius and *S. cyaneus* Linné really represent the European and North American geographical races of one species, while *S. juvencus* Linné is perhaps intermediate between the two.

In addition to these forms the North American *Urocerus albicornis* Fabricius, *U. gigas flavicornis* Fabricius, *Sirex aerolatus areolatus* Cresson and *S. areolatus coeruleus* Cresson, as well as the holarctic *Xeris spectrum* Linné and the European *Urocerus augur* Klug, have been recorded at different times. In addition to Chrystal (1928), see Saunt (1925) for some further records of these captures, and Bradley (1913) for the taxonomy of these insects.

Larix.

Larix decidua Miller was apparently first introduced into Britain about the beginning of the seventeenth century, and *L. krempferi* Lambert (= *leptolepis* Endlich) about 1861 according to Elwes and Henry (II, 387).

Lygaeonematus erichsoni Hartig.

This species has threatened at times to become a serious pest. There were some very bad outbreaks in Cumberland immediately before the war. The adult and larva are described by Cameron (II, 51) and more recently by Hewitt (1908) and others.

Lygaeonematus laricis Hartig.

The first definite reference to this species in Britain, with an account of its larva after Brischke, is given in Cameron (IV, 191-2) as "*Nematus laricivorus* Zaddach". The species is further described in Enslin (p. 508). It is now apparently generally distributed in Britain from Devonshire to

Sutherland. Perkins (p. 305) records that in Devonshire it is sometimes so numerous as to be injurious to the trees.

Lygaeonematus wesmaeli Tischbein.

This species was first recorded as British by Morice (1919) when an outbreak occurred at Arncliffe in Yorkshire. Perkins (p. 305) records it also from Devonshire and tells me he has examined specimens from Buckinghamshire and Essex. I have seen specimens also from Gastell Loch, Glamorgan (Hallett), and Clawthorpe, Westmorland (Miles) and Forest of Dean, Gloucestershire, 1931 (J. C. Robbins). The species is described by Enslin and the larva, after Tischbein and van Vollenhoven (p. 496).

Pachynematus imperfectus Zaddach.

This species was discovered in Britain by Perkins (p. 305) in Devonshire, but I have seen specimens also from Forest of Dean, Gloucestershire, 1931 (J. C. Robbins), Guildford, Surrey (G. C. Champion) and Boxmoor, Hertfordshire, 1933 (Benson). The species is described by Enslin (p. 484) and the larva after Brischke (p. 485).

Platycampus duplex Le Peletier.

This was first recorded as British by Cameron (II, 223-4) as "*Camponiscus apicalis* Brischke" from specimens collected by Billups at Weybridge, Surrey. The larva is described in Enslin (p. 325) after Carpentier. Perkins (p. 298) records the larvae as common in Devonshire, but difficult to rear. I have seen material also from Forest of Dean, Gloucestershire, vi. 1931 (J. C. Robbins); Skiffness, Sussex, 21. v. 1892 (Jenner Coll.); Halton, Buckinghamshire, v. 1929 (Benson); and Babdy, Warwickshire (J. W. Saunt).

Picea.

Picea excelsa Link was, according to Elwes and Henry (v, 1351), first introduced into Britain early in the sixteenth century; on the continent it occurs in various parts of Europe, especially in Scandinavia.

Diprion polytomum Hartig.

Several specimens of this species have been recorded in Berkshire and Hampshire during the last few years. The first records were larvae bred by Miss Chawner (Benson, 1933). A description of the larva after Hartig is given in Enslin (p. 549). A detailed account of the biology of this species is being worked out by Dr K. R. S. Morris, Farnham Royal.

The species has assumed importance in the last few years on account of the devastating outbreak in the Gaspé peninsula of Quebec.

Lygaeonematus abietinus Christ.

This species was first recorded as British by Perkins (p. 305) from Devonshire specimens. In the Morice collection are specimens from Guildford, Surrey (Morice) and King's Lynn, Norfolk (Atmore). I have found the species also myself at Berkhamsted, Hertfordshire, 1926. Mr A. W. Stelfox has sent specimens to Dr R. C. L. Perkins, F.R.S., from Dublin and Wicklow in Ireland. The larva is described by Enslin (pp. 497-8) after Hartig, Brischke and Zaddach.

Lygaeonematus ambiguus Fallen.

This species was known to Cameron (II, 70-1), and there are specimens in his collection from near Glasgow and Strathblane, Stirling; Perkins (p. 305) found it in Devon, but not commonly, and I have also found the species on the borders of Buckinghamshire and near Tring, Hertfordshire. The larva is not yet distinguished from that of the proceeding species.

Lygaeonematus compressus Hartig.

Dr R. C. L. Perkins, F.R.S., has only seen two British specimens, the first captured at Rannoch, Perthshire, vi. 1927 (Harwood), and the second from Dumfriesshire more recently. In addition to these I have examined one female, now in the British Museum, from Laughton, Sussex, 5. vi. 1888 (Jenner Coll.), one male from Lyndhurst, Hampshire, bred from a larva by Miss Chawner, also in the British Museum, and one female, Dingwall, Ross-shire, viii. 1909 (J.J.F.-X., King Coll.). The larva (Enslin, p. 499) is not certainly separated yet from *L. saxeseni* Hartig.

Lygaeonematus saxeseni Hartig.

This species was first recorded as British by Morice (1906, p. 250) and there are specimens in Morice's collection from Kingston, Surrey (Theobald) and King's Lynn, Norfolk (Atmore). Perkins (p. 305) records the species in Devonshire as more numerous than *L. abietinus* Christ. I have found and bred larvae from Buckinghamshire and Hertfordshire, near Tring. The larva is described by Enslin (p. 499) after Stein.

Pinus.

The position with regard to this genus is not so simple. *Pinus sylvestris* Linné var. *scotica* was formerly widely distributed over the British Isles according to Elwes and Henry (III, 576, etc.), but is now confined principally to a few forests in North Britain, such as Black

Wood of Rannoch and the Spey and Dee valleys. In the seventeenth century it was reintroduced into southern England from north British stock, together with other species from the continent.

Specimens of sawflies on *Pinus* in the south of England are possibly alien representatives of species which were at one time native there, but are now native only in north Britain. Several native species of *Diprion* that occur in north Britain have not yet been found in the recent plantations in the south, where *D. pini* Linné and *sertifer* Geoffroy seem to be the only universally common species. Mention should be made of *D. virens* Klug which may be an alien, as, so far, it has only been found in the New Forest, Hampshire (Miss Chawner) and Colchester, Essex (B. H. Harwood).

A key to the British species is given by Morice (1913, pp. 144-5), and further descriptions of the species and their larvae can be found in Enslin (pp. 539-63).

MONOCOTYLEDONES.

Polygonatum.

Phymatocera aterrima Klug.

Although this species is so abundant now in gardens in the south of England, it was apparently unknown to the earlier writers except Curtis (1839), who discovered it near Putney, and who, after describing it as a new species, *Selandria robinsoni*, deals with its habits and life history. Cameron (I, pp. 231-2), who gives a description of the larva, knew only of Curtis' record.

The species is included here on the suspicion that it is alien in origin. The large black flies appear in gardens in May in the south of England almost wherever Solomon's seal (*Polygonatum* spp.) is grown: by mid-summer the plants are often entirely defoliated by the dull grey larva. The striking appearance of the species and its great abundance make it all the more remarkable that the species was so little known in England until the present century. It is not possible to tell where the garden stock of *Polygonatum* originated; it is just possible that it was brought into this country from abroad.

I have found *Phymatocera aterrima* Klug, both adults and larvae, on wild *Polygonatum multiflorum* Moenick in beech woods near Halton in Buckinghamshire; the larvae in this case being very pale in colour compared with the garden ones, probably owing to the denseness of the beech woods where the plant grew. I have not had an opportunity of looking

for it on the other two wild *Polygonatum* species in Britain, and I am not aware of its having been found north of Cambridgeshire in the east or Gloucestershire and Glamorganshire in the west.

CRYPTOGAMEAE.

FILICALES.

Blasticotoma filiceti Klug.

I have already recorded (Benson, 1934) the occurrence of this species in the Royal Horticultural Society's Gardens, Wisley, Surrey, where the larvae were first discovered in 1922 by Mr G. Fox-Wilson, boring in the stems of the ferns, *Athyrium Filix-foemina* Roth., *Polystichum* sp. and *Mattheucia Struthiopteris* Linné. As I then pointed out, the species is very likely to have been introduced, but nevertheless may well occur as a native. A description of the larva and biology can be found in Enslin (p. 627) after Meijere.

Other fern-feeding species, such as *Heptamelus ochroleucus* Haliday, whose larva also bores in the leaf stems, are perhaps sometimes brought into the country from abroad, but undoubtedly occur also as natives.

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AN ACCOUNT OF THE CONSTITUTION AND USE OF AN ATOMISED WHITE OIL—PYRETHRUM FLUID—TO CONTROL *PLODIA INTERPUNC- TELLA* HB. AND *EPHESTIA ELUTELLA* HB. IN WAREHOUSES

By C. POTTER, B.Sc.

(Department of Entomology, Imperial College of Science and Technology.)

(With Plates XXXIII–XXXV and 4 Text-figures.)

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INTRODUCTION.

THE control or reduction of infestation of agricultural produce by insects is commonly directed towards the destruction of these insects in the produce itself, mainly because it is the vendors or users of the product who are most concerned with its infestation. Equally important is the control of the insects in the premises, ships or warehouses, in which the produce is stored. This, however, is not sufficiently realised and the problem of the infestation of clean or moderately clean produce by the insects which inhabit ships and warehouses has been too much neglected.

The reason for this neglect is that the life history of the insects in the warehouse has not been worked out before, and therefore the fact that a large proportion of them spend one stage of their existence in the fabric of the building was not known.

In the ordinary course of trade, produce subject to insect attack may be infested in the country of origin, during transport and during storage in the importing country.

The work to be described was carried out with a view to protecting stocks of Australian dried fruits stored in London. These stocks had faced the risk of infestation in the country of origin, and also during transport from the producing area to the London warehouse. On arrival in London the fruits are fumigated in barges, but until recently the efficiency of this barge fumigation was not very high, and consequently a number of insects survived, were carried into the warehouses with the fruits, and became established there.

The two insect pests of dried fruits causing damage in this country are *Plodia interpunctella* Hb. and *Ephestia elutella* Hb. Life-history studies made in the warehouse, coupled with inspection work there, showed that the full-grown larvae of these two species normally over-winter in the fabric of the warehouse and emerge as moths the following year to infest any goods that may be present. A resident population is thus present in the warehouse which is not necessarily eliminated by removing or cleaning any infested goods present. A considerable proportion of the total amount of infestation of goods in this country appears to arise from infested premises.

As the result of the bringing into operation of a very efficient system of barge fumigation, it may now be safely assumed that all infestation present in Australian dried fruits on arrival is killed before the fruits are taken into the London warehouses. The problem remained to clear the warehouses of infestation.

Fumigation to ensure complete destruction of *Plodia interpunctella* Hb. and *Ephestia elutella* Hb. in the warehouse is not always practicable. Some of the problems involved in the fumigation of warehouses are described in the papers of Drs A. B. P. Page and O. F. Lubatti of this department (16, 17), and it is sufficient to state here that it has not so far proved practicable to obtain complete destruction of the over-wintering insect in the type of building in which the dried fruits are stored. It is necessary to fumigate in the winter, since the sheds are full of fruit at other times and the absorption and adsorption of fumigant by the stored goods when added to the losses due to leakage would greatly increase the cost of fumigation.

Because fumigation cannot, except at a prohibitive cost, get rid of the resident population, the material and methods described in this paper were developed in order to do so. These methods take advantage of the fact that as long as clean goods are being brought in, the infestation occurring in the English warehouse, whether the source is the premises or the goods themselves, is cyclical, and the stage in its life

cycle reached by the insect can be predicted within narrow limits for any given period of the year. In order to make this prediction it is necessary to know the life history of the insect under the particular conditions where the control method is to be applied. The primary object of the methods here described is to kill the moths as they emerge in the spring before they are able to lay eggs on the goods stored in the warehouse.

In addition, on any occasion where goods have become infested these methods can be used to reduce the infestation and prevent its spread to other goods and to the premises. Apart from that relating to fumigants there appears to be no published information on insecticides used specifically against stored products pests. The material and methods described here had therefore to be worked out from first principles. As it was expedient to obtain practical results in the field as soon as possible, parts of the investigation of less immediate practical importance are incomplete. These will be developed later on.

Although much information is contained in the literature relating to insecticides applied to plant pests, very little of this is directly applicable to the control of the pests of stored products for a number of reasons, such as the known high resistance of stored products' insects to insecticides and the risks which are run of tainting foodstuffs or other materials such as tobacco. Only a few of the works which have been consulted are referred to in the text and in the short list of references at the end. Especial mention should be made of the papers of Dr Tattersfield and his co-workers (24, 25, 26, 27) which provided valuable data on the pyrethrins. The monograph on pyrethrum flowers by C. B. Gnadinger⁽⁴⁾ also proved very helpful, both practically and theoretically. I am indebted to Mr W. E. Edmonton of Messrs Stafford Allen and Sons for a number of suggestions and practical help.

This investigation was started at the suggestion of Prof. J. W. Munro of the Imperial College of Science and Technology in whose department and under whose direction it was carried out. The work was done on behalf of the Australian Dried Fruits Board who provided the necessary funds. My thanks are due to Mr J. J. S. Scouler, Member and Secretary of the Board in London, for the many facilities given for field work in the Board's warehouses, and for the keen interest he has taken in all phases of the work.

LIFE HISTORY OF *EPESTIA ELUTELLA* Hb. AND *PLODIA*
INTERPUNCTELLA Hb. IN THE LONDON WAREHOUSES.

The following account of the life history of *Ephestia elutella* Hb. and *Plodia interpunctella* Hb. is based upon data obtained from experiments in the London warehouses, correlated with observations made on the insects under natural conditions there. The life history has not been studied before in the London warehouses, and a detailed account will be published at a later date, showing how various conditions of storage affect it.

Both *Plodia interpunctella* Hb. and *Ephestia elutella* Hb. normally pass the winter in the English warehouse as full-grown hibernating caterpillars "spun up" in cocoons in some crack or crevice. A few isolated observations indicate that young larvae are able to survive the winter in the food material, but this rarely occurs.

The usual type of warehouse in this country is a several-storey building with brick walls and wooden floors and ceilings. In this type of building there is one generation of the insect in the year in any floor except the top floor. Three sets of experiments were carried out during 1931-2 on the second floor of a warehouse of five floors. The average periods from egg to adult for *Ephestia elutella* Hb. were 357 days, 367 days and 366 days respectively. The temperatures prevailing during these experiments are shown in Table I.

Table I.

Temperatures for the months during which the life history was studied under wharf conditions.

Month	Max. °F.	Min. °F.	Average °F.	Month	Max. °F.	Min. °F.	Average °F.
1931				1932			
July	67	62	63.3	January	51	43	47.1
August	67	58	63.0	February	45	37	40.8
September	62	53	58.2	March	49	40	42.6
October	60	46	55.8	April	52	44	48.1
November	52	46	51.2	May	60	48	53.5
December	51	43	47.4	June	66	54	59.0
				July	72	60	65.6

Under these conditions of storage the hibernating caterpillars begin to pupate in May and the moths begin to emerge early in June, the pupal period varying from 3 to 5 weeks. Emergence continues until the end of July. The peak of emergence is about the beginning of that month. The majority of *Plodia interpunctella* Hb. moths emerge shortly after those of *Ephestia elutella* Hb., but the periods of emergence overlap to a considerable extent. The moths emerge and pair at night.

The eggs are laid either on the food material or between the boxes or sacks in which it is contained. The incubation period of the egg varies but is about 7 days in June and July.

The young larvae after hatching eat their way into the block of fruit and sometimes enter individual fruits. A badly infested 56-lb. box of fruit will have young larvae distributed throughout it.

Towards the end of August the first caterpillars become fully grown. Most of these leave the fruit and migrate in search of a suitable crevice in which to open a cocoon. During this migratory phase a large number of insects reach cracks and crevices in the fabric of the warehouse and establish themselves there for the winter. The majority of English warehouses offer many suitable hibernating places for the insects. Usually migrating caterpillars are found until about the end of November. The main migration of the larvae of *Ephestia elutella* Hb. takes place shortly before that of *Plodia interpunctella* Hb.

During their migratory phase the larvae of both species are negatively geotropic, negatively phototropic and will enter any suitable crack or crevice to spin their cocoons.

A few larvae do not migrate but remain in the food material. The larvae of *Plodia interpunctella* Hb. do this more than those of *Ephestia elutella* Hb. A number of the larvae spin their cocoons either inside the containers or between them.

The life history just outlined applies to multi-storeyed warehouses. The dried fruit is stored in London in large single-storey sheds. In the main body of these sheds the life history follows closely the course just outlined, but, under the conditions of high maximum temperatures prevailing in situations close underneath the roofs of these buildings, the moths emerge early, and there are two generations in the year. Emergence of moths begins about three-quarters of the way through May and the resulting generation takes from 2 to 3 months. The second generation of moths appear in August and September, these moths again lay eggs and the caterpillars that hatch from these eggs hibernate during the winter in the usual way.

In a single-storey warehouse all gradations occur between the warm conditions such as are found underneath the roof and the relatively cool conditions at ground-level. These latter approximate to those found in the middle floor of a several-storey warehouse. This gradation causes a great deal of overlapping of the various stages and makes control difficult.

THE INSECTICIDE.

The properties of a liquid insecticide for use on stored foodstuffs and other goods in warehouses so differ from those of a plant or household spray that none of these could be used.

A spray for use on stored products must possess the following properties:

- (1) It must be non-poisonous to human beings.
- (2) It must be non-inflammable.
- (3) It must impart no taint or cause any deterioration of the product.
- (4) It must be available in commercial quantities at a price which is economically practical.

The only suitable substance of recognised toxicity readily available at this time was pyrethrum. At the beginning of the investigation it was found that the fully grown larvae of both *Plodia interpunctella* Hb. and *Ephestia elutella* Hb. were very resistant to pyrethrum; a petroleum oil was therefore incorporated in the insecticide in order to increase its efficiency. A formula was evolved for an aqueous spray which consisted of an emulsion of petroleum oil and pyrethrum extract, with stiffened sulphonated castor oil as the emulsifier.

This spray proved to be moderately effective on the full-grown larvae of the two species of insect, and further work on it is still in progress.

It was then found that the spray would have to be used to prevent moths emerging from the fabric of the warehouse from reaching the stored goods and, as an aqueous spray is quite ineffective against such an attack, it was decided to use the concentrated insecticide in an atomised form. When the insecticide is used in this way both water and the emulsifier are no longer necessary.

The insecticide finally used consisted of a preparation of pyrethrins I and II in a highly refined white oil of the following specification:

Specific gravity	0.862
Flashpoint closed	320° F.
Flashpoint open	335° F.
Viscosity Redwood 1 at 70° F.	118 sec.
Pour test	30° F.

An oil having this specification may be obtained from the firm of Shell Mex and B.P., Ltd., under the title of Shell Oil 24210. It is transparent, colourless, tasteless and odourless, and resembles medicinal paraffin. It does not taint and is non-volatile. All the petroleum oils

used in plant sprays taint to some extent, and those used in household sprays are, of necessity, volatile.

In practice the insecticide was made up by diluting with Shell Oil 24210 a preparation containing about 6.5 per cent. pyrethrins I and II in a mineral oil of similar specification to that given above. The pyrethrum preparation used was made by Messrs Stafford Allen and Sons under the name of Pyrethrum Extract M. 225.

When a technique for atomising this material in the warehouse in order to kill moths had been worked out, it was found that the process could also be readily adapted for use against the full-grown migrating caterpillars.

The atomised pyrethrum-white oil solution is better than an aqueous spray for several reasons. First, the considerable quantities of water which have to be used with an aqueous spray have little or no effect on the insect, also they are liable to cause deterioration of the goods in storage. There is considerable evidence to show that an aqueous spray used in 1932 set up serious fermentation in cases of dried fruits. The use of an atomised spray reduces the risk of this type of damage because of the small quantities of material required; it also facilitates transport for the same reason.

Secondly, the penetration of an atomised spray is greater than that of a water spray applied by means of a pressure spraying machine. The particles of concentrated insecticide are carried by the compressed air into places which are inaccessible to a jet of liquid whatever type of nozzle is used.

Another advantage of this pyrethrum in white oil solution is the low volatility and high viscosity of the white oil which is used as the carrier. Field experiments, details of which are given later on, show that, when the atomising method is used with this type of oil carrier, a light protective film is formed on exposed surfaces. This film is not visible to the naked eye, but moths and larvae which come into contact with it are killed. Further experiments, the full details of which are not yet available, confirm the presence of this film and show that under some conditions it will remain effective for several weeks. The formation of this protective film is a valuable feature of the material and method.

The insecticide has two disadvantages which are due to the high viscosity of the oil used. First, it is difficult to atomise, so that a relatively large amount of compressed air is required to atomise a small quantity of spray. The second disadvantage is the probable loss of toxicity due to slow penetration of the oil, although there is no definite

evidence on this subject. Gnadinger (4), p. 163) states that the relative toxicity of sprays made from different fractions of petroleum with the same pyrethrin content will vary according to the type of sprayer used and more or less independently of the physical constants of the oils used.

The insecticide has been tested on dried fruit and tobacco. It does not taint these products and is therefore suitable for use in warehouses containing them. It is now extensively used in warehouses containing dried fruits, and it is also being used in a warehouse containing stored cacao.

Under normal circumstances, owing to the high flash-point of the oil there could be no risk of fire, but there was the possibility that the material was readily inflammable in an atomised state.

In order to decide this point Dr A. B. P. Page carried out a test of the inflammability of the air-atomised spray.

The liquid consisted of one part of the pyrethrum extract and three parts of the oil by volume, and was atomised through a standard nozzle at 75 lb. pressure in exactly the same manner as in commercial use. Various settings of the nozzle were tried but without affecting the inflammability.

Different measured amounts of the oil were sprayed into a cubical airtight chamber of aluminium, the sides of which are 4 ft. long, the capacity thus being 64 cu. ft.

The concentrations of spray in c.c. per 1000 cu. ft. used were 4, 8, 16, 32, 64, 128, 192, 256, 640, the actual concentration used at Millwall Wharf being 33 c.c. per 1000 cu. ft.

The following sources of ignition were applied to the mixture in the chamber immediately after atomisation:

- (a) A stream of sparks $\frac{1}{2}$ in. in length produced by a magneto.
- (b) An air-coal gas flame about 8 in. long from a blowpipe. This was played on to a small iron surface suspended by a wire, this raising it to bright incandescence.
- (c) The combustion of 12 in. of magnesium ribbon.

During each test the air in the chamber was heated by the blowpipe to between 30 and 35°C.

In order to determine whether there was any possibility of igniting the spray as it left the nozzle, a stream of the spray was directed at various angles on to the blowpipe flame and on to the hot-iron surface. It was also allowed to play on to the spark gap while the sparks were passing.

In no case was there the slightest indication even of ignition, still less of the propagation of any flame or explosion through the air-spray mixture.

Evidently the finely divided spray does not form a combustible mixture with air as ordinarily understood, hence the terms upper and lower explosion limits, or limits of inflammability, are not applicable to it.

TECHNIQUE.

The technique of application of atomised sprays is comparatively little developed.

Le Pelley (13, 14) describes a small hand sprayer used to atomise kerosene-pyrethrum extracts in tents against coffee pests. Except for the small atomisers used with household insecticides, this was the only account of a method of atomising an insecticide on a practical scale when the apparatus and methods described in this paper were first used in the warehouse.

Lamimam (11) has since described the application of a kerosene-pyrethrum extract against grape-leaf hopper by means of a paint spraying outfit, and Parker (19) gives a general account of a special machine for atomising oils against plant pests. An article has been published in *Soap* (1) describing various types of small steam sprayers used for atomising household insecticides. In this article mention is made of the use of live steam to atomise insecticides in warehouses, but there is no account of any apparatus and no details are given. It is not possible therefore to form any definite opinion of its value, but the use of live steam in warehouses containing products such as dried fruits would almost certainly result in serious damage to the goods in store.

I have devised two types of apparatus to atomise the insecticide, an atomising gun and an atomising unit. The unit works on the same principle as the gun but is designed to save labour in certain special circumstances. It should only be used in conjunction with the gun. Both these types of apparatus are worked by compressed air which is supplied by an air compressing machine.

Plate XXXIII, fig. 1, is a photograph of a complete apparatus using four guns. When units are being used, three of the guns are replaced by two units. This apparatus is the largest that is conveniently portable by man-power.

It was used for this experiment because twenty sheds, each of an average cubic capacity of 185,000 cu. ft., had to be treated. For this purpose two of these outfits were required.

In this country warehouses are usually divided into sections of considerably less cubic content than each of these sheds, and in these circumstances an air compressor working one or two guns is sufficient.

The warehouses which were first treated were single-storey sheds covering a large area of ground where it was necessary to use a portable air compressor. A large number of the warehouses in this country have several storeys. Here it is more convenient to instal the compressor on one of the floors. The compressed air is led to all the floors through a vertical iron pipe, connected to the air compressor and running from the top to the bottom of the warehouse. The vertical pipe has a tap on every floor. It is then only necessary to transfer the hose and the atomising apparatus from floor to floor. A system of this kind has been installed in one warehouse and has proved very satisfactory.

The following is a description and specification of the air compressor shown in Plate XXXIII, fig. 1. It is a twin-cylinder machine, each cylinder having a 4 in. bore and a 3 in. stroke which gives a piston displacement of 33 cu. ft. of free air per minute when running at 750 revolutions per minute. It is air cooled. The air is compressed into a mild steel air receiver, 4 ft. long and 15 in. in diameter. The air receiver is fitted with a delivery tube containing six cocks for the reception of the hoses of the atomising apparatus.

The compressor is driven through multiple vee belts by a 4 H.P. D.C. motor working off 440-volt mains. The whole apparatus is mounted on four rubber-tyred wheels, of which the front two are on a swivel attached to a drawbar, so that the machine is transportable.

This compressor works four atomising guns, or two atomising units and one gun, at a pressure of about 65 lb. per sq. inch.

The essential property of an air compressor for this work is that it should provide a continuous supply of sufficient compressed air, at a pressure of from 35 to 70 lb. per sq. in., to work the atomising apparatus required.

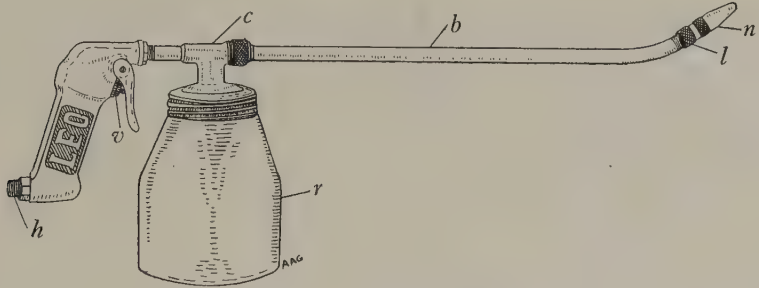
The atomising guns.

The type of atomising gun used is shown in Text-fig. 1. It has not, to my knowledge, been used before for this particular purpose, and the reasons for its selection are given after the description of its construction and mode of action.

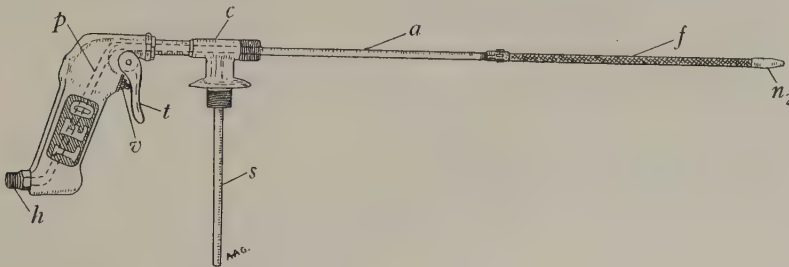
The mechanism of the gun is shown by Text-figs. 2 and 3. Text-fig. 2 shows the path of the air through the gun and illustrates the way in which the liquid is sucked up.

The compressed air enters the gun at the hose connection (*h*), passes up through the air passage (*p*), through the air tube (*a*) and the flexible extension of the air tube (*f*), and out through the bore of the inner cone (*n*₂).

The passage of the air may be stopped by means of the trigger (*t*) which operates the valve (*v*). The structure of the valve is not shown in Text-fig. 2 for the sake of simplicity.



Text-fig. 1. Sketch of complete gun. *b*, barrel; *c*, connecting piece; *h*, hose connection; *l*, lock-nut; *n*₁, outer adjustable cone of nozzle; *r*, reservoir; *v*, trigger valve.



Text-fig. 2. Sketch of gun without barrel and reservoir. *a*, air tube; *c*, connecting piece for air tube and suction tube; *f*, flexible extension of air tube; *h*, hose connection; *n*₂, inner cone of nozzle; *p*, air passage; *s*, suction tube; *t*, trigger; *v*, trigger valve.

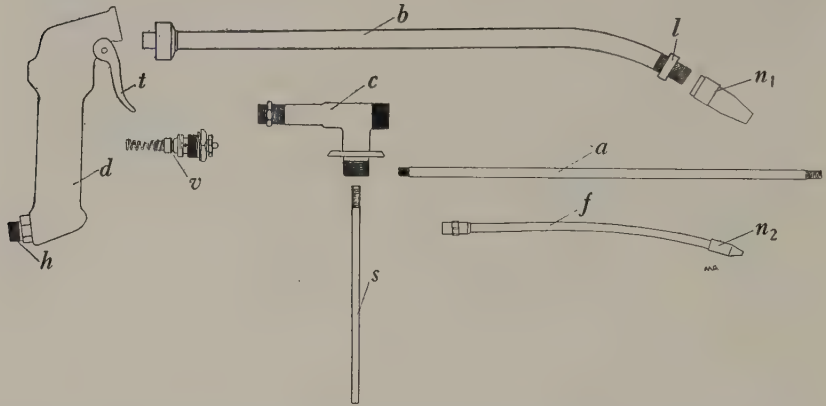
The liquid is sucked up through the suction tube (*s*) which reaches to the bottom of the reservoir (*r*), it then passes into the annular space between the barrel (*b*) and the air tube (*a*) and the flexible extension of the air tube (*f*); finally it passes out between the cones (*n*₁) and (*n*₂).

The flexible extension (*f*) allows the nozzle to be turned at an angle without making the gun any more difficult to dismantle. This tube, however, is sometimes responsible for faulty atomisation because it is liable to leak. Text-fig. 3 which shows the constituent parts of the gun also shows the way in which the outer cone (*n*₁) of the nozzle may be

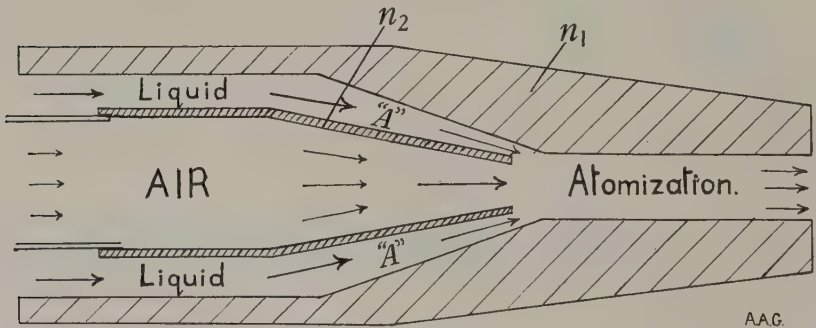
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screwed up or down to adjust on the inner cone (n_2). In addition it shows how the adjustment is fixed by means of the lock-nut (l).

The diagrammatic section of the complete nozzle (Text-fig. 4) shows in detail the method of atomisation. The compressed air passes up



Text-fig. 3. Diagram to scale of component parts of gun. a , air tube; b , barrel; c , connecting piece for barrel and suction tube; d , butt; f , flexible extension of air tube; l , lock-nut for outer cone of nozzle; n_1 , outer adjustable cone of nozzle; n_2 , inner cone of nozzle; s , suction tube; t , trigger; v , trigger valve.



Text-fig. 4. Longitudinal section of complete nozzle ($\times 3$). n_1 , outer cone of nozzle; n_2 , inner cone of nozzle.

through the bore of the inner cone (n_2) and through the aperture of the outer cone (n_1). A reduced pressure is created at the apex of the inner cone which causes the liquid in the annular space ("A") to be sucked out between the external surface of the apex of the inner cone and the internal surface of the outer cone. The alteration of the distance between

these two surfaces varies the quantity of material emitted from the gun and the degree to which it is atomised.

The nozzle of this type of gun can be adjusted over a very wide range of degrees of atomisation. When the outer cone is screwed down on the inner cone very fine atomisation is produced; as the outer cone is screwed away from the inner cone, the atomisation grows coarser until, with the outer cone screwed right away from the inner cone, the insecticide is scarcely atomised at all.

The insecticide liquid contains a considerable amount of resinous material which is liable to accumulate in the gun and cause a blockage. When a blockage occurs in this type of gun it is almost invariably at the nozzle and can be quickly cleared by unscrewing the outer cone a few turns.

The model used is manufactured for the paraffin cleaning of cars.

The type of nozzle usually found in the atomising guns that are used to atomise paint and water consists of a hollow-ground cone with a hole bored down the centre from which the liquid is sprayed out.

The atomisation is accomplished by means of compressed air issuing from a fixed annular space round the tip of the cone. Guns with this type of nozzle are designed for a gravity feed.

The disadvantages of this type of nozzle compared with the type used are: first, that it is gravity fed and the gun must therefore be held upright; secondly, that blockages may occur very frequently and cannot be cleared easily; thirdly, this type of nozzle cannot be adjusted for the very wide range of atomisation obtainable with the type of nozzle used.

The quantity of any given liquid atomised by one gun varies with the adjustment of the nozzle and with the air pressure. The degree of atomisation also varies with these two factors.

In order to decide which pressure gave a sufficiently even atomisation, some tests were carried out with the insecticidal material using air pressures ranging between 20 lb. per sq. in. and 90 lb. per sq. in. It was found that with the nozzle adjusted to give the degree of atomisation used against moths a pressure of 65 lb. per sq. in. applied an even coating of material to a square of white paper at a distance of 4 ft.

The nozzle was set to give the degree of atomisation necessary for use against the moths, and various pressures were tried to determine what pressure would atomise the greatest quantity of insecticide in a given time. It was found that from 60 to 100 lb. per sq. in. there was no appreciable change in the amount of liquid atomised. Below 60 lb. per sq. in. the amount of liquid atomised was not so great. From this date the working pressure was fixed at 65 lb. per sq. in.

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At this pressure the gun will use about 8 cu. ft. of free air per minute. Although a pressure of about 65 lb. per sq. in. has been found to give the maximum quantity of spray with the requisite degree of atomisation, the gun may be adjusted to work satisfactorily with the air pressure as low as 35 lb. per sq. in.

Because there is no direct method of measuring the degree of atomisation obtained that can be used in commercial practice, an indirect method was adopted.

It was found that when the nozzle was set so that at an air pressure of 65 lb. per sq. in. one gun atomised 500 c.c. of liquid in 45 min., the degree of atomisation was such that the mist of insecticidal material hung for 2 hours without clearing appreciably. This type of mist was found to be satisfactory as an insecticide. The degree of atomisation obtained with the guns was therefore standardised by measuring the quantity of material atomised in a given time under given conditions of air pressure. During the course of the experiment various nozzles were tested in order to compare the degree of atomisation obtained. This comparison was made indirectly by comparing the rate at which the mists produced by the different nozzles settled.

The figures given for air pressures and standards of atomisation cannot be accepted as final, because it is necessary to do a considerable amount of more detailed work on the subject. In the meantime they have been found to give satisfactory results in practice.

The atomising units.

The atomising process was tried out in 1933 using an air compressor and four guns. It was found to give good results, and it was decided to use it systematically in 1934. This meant that a very large air space varying from about 1,830,000 to 2,765,000 cu. ft. had to be filled with mist every 24 hours for a period of over 2 months. The cost of the labour necessary to do this with guns alone would have been very high. In order to reduce the labour cost an atomising unit was designed to fill the larger spaces in the shed with mist. This unit does the work of two guns and remains in the same position throughout the process.

It consists of six nozzles supplied by a common air line and a common reservoir.

The nozzles work on the same principle as those of the atomising guns and are arranged round the circumference of a circle so that they form a mist in every direction radiating from the unit.

Plate XXXIV, fig. 1, shows the first unit which was designed. In

this apparatus a bypass with a reducing valve and gauge was connected between the air line and the reservoir. By means of this device a pressure could be applied to the liquid in the reservoir to force it up to the level of the nozzles.

This experimental unit was designed to test whether a pressure feed offered any advantage over the suction feed for a particular nozzle. It was found that there was no advantage in pressure feeding if the reservoir was not appreciably deeper than 6 in. The subsequent units, one of which is shown in Plate XXXIV, fig. 2, were therefore designed for suction feed.

The nozzle (Plate XXXIV, fig. 3).

This works on the same principle as that of the atomising gun, but the air consumption and atomising surface are reduced.

Plate XXXIV, fig. 4, shows a transverse section through the complete nozzle and its body. Air enters the body of the nozzle (*B*) through the aperture (*A*) and issues through the bore (b_2) of the inner cone (n_2). The amount of air passing through may be regulated by the screw (*s*). The liquid enters the spray body through the aperture (*L*). It passes between the outer cone (n_1) and the inner cone (n_2) and is then broken up by the compressed air to issue in an atomised state from the bore (b_1) of the outer cone (n_1). The outer cone (n_1) is first set to give the required degree of atomisation and then held in position by means of the lock-nut (*N*). Plate XXXV, fig. 1, is a photograph of the nozzle with the outer cone and lock-nut removed. Plate XXXV, fig. 2, shows the unit with the reservoir removed. The air ring (*A*) is connected to the compressed air supply through the tap (T_A). The air ring supplies all six nozzles as illustrated by Plate XXXV, fig. 3, where the disposition of the nozzles is also shown. Each nozzle has a separate feed tube (*T*, Plate XXXV, fig. 2) which supplies the liquid from the reservoir. Every part of the unit may be detached for the purpose of cleaning.

The following are the important measurements of the essential parts of the unit (Plate XXXIV, fig. 2):

Reservoir $\frac{1}{8}$ in. welded mild steel: $8\frac{7}{16}$ in. internal diam. and $6\frac{1}{8}$ in. internal depth.

Air ring of $\frac{1}{16}$ in. copper tubing: $\frac{9}{32}$ in. internal diam.

Suction tubes of $\frac{1}{16}$ in. copper: $\frac{7}{32}$ in. internal diam.

Height from tip of suction tube to nozzle aperture: 8 in. (approx.)

Air tap: $\frac{1}{4}$ in. aperture.

The nozzle:

Inner cone (n_2): base 0.310 in. diam.; tip 0.120 in. diam; height 0.350 in.

Bore of inner cone (b_2): 0.046 in. diam. and 0.25 in. long.

Bore of outer cone (b_1): 0.135 in. diam. and 0.375 in. long.

Air inlet in spray body (a): $\frac{11}{84}$ in. diam.

It is hoped to improve considerably on the design of the units. The nozzles were liable to choke and the atomisation given was not as good as that produced by the guns, so that staining occurred in the immediate neighbourhood of the unit. Alterations to the nozzles and air leads should overcome these defects and experiments on these lines are in progress.

The method of application of the atomised insecticide varies with the stage of the insect. A different method is used against moths from that used against the fully grown migrating larvae.

Technique against moths.

A cloud of finely atomised particles of insecticide is formed by means of the guns or the units and a gun. This mist should hang for a period of from 1 to 2 hours. A concentrated mist lasting for 2 hours can be produced with the apparatus described. No data are available on the relative effectiveness of particles of insecticide of different degrees of fineness, and it is hoped to do some work on this point. At the end of the operation the mist should be slightly more concentrated at the roof of the building than at ground-level.

The compressed air used for atomising the insecticide has the additional effect of creating air currents which carry the mist to all parts of the warehouse.

If the moths present are not flying the insecticide, which has an irritant effect, causes them to fly. While flying through the mist, particles of insecticide accumulate on their bodies and cause a partial paralysis which brings them to the floor, from which they can take only short convulsive flights. At a later stage the paralysis becomes more marked until the insects are only capable of a quivering movement and finally die. The settling of the mist increases the concentration of the insecticide at ground-level, and if the moths brought down remain in exposed positions it will kill any individuals that have only been slightly affected. It was found, however, that moths brought to the ground usually either attempted to get out of the warehouse by crawling under the doors or any other exit, or else they crawled underneath any available shelter.

It is therefore necessary to spray these places with a coarse spray at the beginning of the operation. In this way a film of insecticide is formed and moths that attempt to crawl away get coated with the film and die. If there is time at the end of the operation it is advisable to spray these situations again.

The time taken by the insecticide to act varies considerably. The factors influencing this action and their relative importance are unknown. The concentration of the insecticide, the degree of atomisation, the position of the insect in the warehouse and the temperature, are probably the most important. Under spring and summer conditions with the dosage and method of application used, the first moths were brought to the ground about 20 min. after the commencement of the process. It is not possible, however, to give an average period from field observations. There was a wide variation in the time between the beginning of the atomisation and the death of the insect. This was probably because the amount of insecticide settling on the body varied with the individual. In no instance when an affected moth was collected and kept under observation did it ever recover.

The process, to be effective, must be practised systematically throughout the period of moth emergence.

It is shown in the section dealing with the biological tests that observation alone gives very little indication of the number of moths present in a warehouse, and the practice of spraying only when moths are seen is quite useless.

Not enough work has been done to determine the maximum interval between atomisations which is safe; it is, therefore, necessary at this stage to treat the warehouse every 24 hours during the period of moth emergence to ensure control of the moths.

The method of application and the dosage.

The following description applies to the warehouses in which all the experimental work was done, but with slight modifications it is generally applicable.

These warehouses consist of single-storey sheds with an average capacity of 185,000 cu. ft. When full of goods, in this instance dried fruits, the free air space is about 91,500 cu. ft., the cases of fruit occupying 93,500 cu. ft. The free air space in the sheds containing lesser quantities of fruit can be calculated from this data. Twenty of these sheds required treatment.

Before beginning the process all the doors are closed and any large apertures such as big ventilators are stopped up. Small apertures, such as those occurring between the bottom of doors and the floors, do not lead to significant losses unless there is a considerable draught through them which draws the mist out.

The air compressor is left outside the building because the fire insurance authorities do not allow it inside, unless the motor working it is totally enclosed. The totally enclosed type of motor is more expensive than the normal type and is also heavier and less efficient. It is therefore usually better to have the compressor outside and conduct the compressed air to the place required by means of pipes or hoses.

Plate XXXIII, fig. 2, shows the air compressor in position with the hoses leading into the warehouse through a hole drilled in the iron doors.

All the earlier work was done by means of guns alone; this method is the one most generally applicable and is the most efficient. The guns are taken to the end of the warehouse remote from the compressor, and the atomisation is commenced.

Four guns were used and three of the operators kept abreast and were spaced evenly across the width of the shed. These three worked back towards the air compressor, their rate of progress being so timed that the mist was evenly distributed throughout the shed. The fourth operator sprayed all the places in which moths would be likely to take refuge when brought down by the mist. The three guns producing the mist should be moved about continuously so that the mist is directed to all parts of the shed. Particular attention must also be given to any narrow spaces such as those between the stored goods and the wall, because not only are these situations difficult to reach, but a considerable proportion of the moths are usually found there. It is further necessary that considerably more mist should be directed into the roof of the building than elsewhere in order to compensate for the settling of the atomised particles.

It is essential when producing a mist that the nozzle of the apparatus is not directed at any surface within a distance of 4 or 5 ft. because the atomised material will condense on the surface and be wasted.

Later, when it was desired to save labour, two units and one gun were used. The units were placed at a distance of one-third and two-thirds respectively from the end of the shed where the air compressor was placed. They were as near the roof as possible and were equidistant from the sides of the warehouse. The gun was used to fill up any spaces

which were not reached by the mist produced by the units and also to spray any places at ground-level likely to shelter the moths.

The dosage generally varied between 2.5 and 3.5 c.c. per 100 cu. ft. of free air space. It may have been higher or lower in a few instances, these discrepancies being caused by the variation in the size of the sheds and the amount of fruit contained in them.

The dosage during the process was measured by timing. The results were verified by ascertaining the quantities of material present in the reservoirs of the apparatus at the beginning and the end of the operation. It was found that with a given setting of the nozzle and a fixed air pressure the amount of material atomised in a given time is approximately constant.

The amount of material atomised in a given time for the required setting of the nozzle was first measured and all calculations of dosage were worked out from this. It was found that with four guns approximately 2000 c.c. of liquid could be atomised in 30 min., and that with two units and one gun approximately 2500 c.c. could be atomised in the same time. The atomisation produced by the units is not as satisfactory as that of the guns and therefore the mist does not hang so well. The measurement of the dosage by timing during the operation is not very satisfactory because there is no absolute standard of atomisation; also, the nozzles are liable to get choked. It is therefore intended to fit liquid gauges on the apparatus so that the quantity of material atomised can be read at any given moment.

The strength of the material used is about 1.6 per cent. total pyrethrins in white oil.

The concentration of the mist and the strength of the material were decided on after some preliminary tests had been done in the laboratory and some observations had been made under working conditions in the warehouse. The figures thus obtained cannot be regarded as final, but they give satisfactory results in practice. It is hoped that it will be possible to carry out a series of tests on the effect of various concentrations of mist, each concentration being tried with material of several different strength of pyrethrins. In the meantime the limiting factors in practice are time and compressed air supply. It is very probable that it would be better to give a larger dosage with a material of less pyrethrin content, but this would necessitate a longer time for the operation or else an increased air supply.

In the experiments described it was not possible to increase the dosage, since this could not be done without considerably increasing the appa-

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ratus and consequently increasing the cost of the labour required to work it. Where it is practical the figures given here for concentrations of mist should be doubled whether the pyrethrin content of the material is reduced or not.

Technique against caterpillars.

Before giving an account of the methods adopted in spraying the larvae of *Ephestia elutella* Hb. and *Plodia interpunctella* Hb. it is necessary to state clearly when this method may be usefully applied and to realise its limitations.

The method is mainly useful in stopping the spread of infestation and preventing reinfestation.

The larvae of the two species of moths only come out of the fruit and expose themselves to the action of sprays when they are fully grown. Spraying is therefore of no use for goods infested with the early stages of the insect. In addition goods infested with fully grown caterpillars cannot be completely cleaned. This can only be done by means of fumigation.

Spraying is used to prevent infestation in three ways. First, when infested goods are stored next to clean goods the fully grown larvae may crawl out and infest the clean goods. By spraying the infested goods most of the wandering larvae are killed and so the spread of infestation is prevented. Secondly, when infested goods are stored in clean premises, they are sprayed to kill the full-grown wandering larvae and these insects do not reach the premises where they normally would over-winter. Spraying in this instance prevents the possibility of clean goods being infested by moths resulting from these caterpillars. Thirdly, when goods infested with caterpillars are taken away either to be fumigated and returned or to be replaced by clean goods, it is necessary to spray the site after the goods have been removed. In this way any larvae that have dropped off during the process of removal are killed. If this is not done the insects left behind are liable to infest any goods placed on that site.

Spraying against larvae is done by means of an air compressor and guns. Air pressures ranging between 30 and 65 lb. per sq. in. are suitable. The greater the air pressure the farther the spray will carry but the larger the proportion of material wasted.

Where the caterpillars are crawling over exposed surfaces no special technique is required. The nozzles are adjusted to a degree of atomisation that does not stain the surface, but is as coarse as possible consistent with an even coating of the surface. In this way the maximum wetting

effect is produced. The caterpillars are then sprayed as by a direct contact spray.

When the larvae are present in a pile of goods a special technique has to be adopted because a considerable number of the larvae are not exposed on the surface, but are crawling about in the interior of the pile. The nozzle is adjusted to give a fine degree of atomisation and the interstices of the pile are filled with a fine mist by inserting the nozzle in any suitable cracks. The fine mist penetrates the pile and has the effect of driving out the larvae. The subsequent treatment depends on the circumstances. Where the goods are stored in wooden cases which may be heavily sprayed without damage, the nozzles are then adjusted to give a coarse atomisation and the outside of the pile is heavily coated with material. The larvae which are driven out from the interior of the pile pick up some of the insecticide while crawling over the outside and are thus killed.

When the goods are stored in sacks or fragile cases they should be left for about 20 min. after the introduction of the fine mist; by this time most of the larvae from the interior have reached the surface. The exterior of the pile is then sprayed with a slightly coarser atomisation. The amount given is adjusted so as not to damage the goods by excessive wetting.

A large proportion of the affected caterpillars fall off the pile, so that the floor round the base of the pile should be heavily coated with spray liquid. This will kill those larvae which have only received a light dose before they fall off the pile.

By this method goods infested with fully grown caterpillars can be rendered fairly clean, but there is always some residual infestation, either because some of the larvae have already spun cocoons or because they have remained inside the containers where they cannot be reached by the mist.

Where the original infestation is slight, the residual infestation may be almost negligible, unless the goods are to be kept in storage sufficiently long for the insects to breed. Where, however, the original infestation is heavy, there may be a considerable residual infestation, but it is in such a condition that it is not likely to spread.

A concentration of about 0.813 per cent. total pyrethrins in white oil was used and found to be effective in practice. The effect of the spray, however, appeared to vary with the time of the year. The factors affecting the variation are discussed in the section dealing with the biological tests.

So far as I know this insecticidal material and method of application

constitute the only spraying technique which is known to be effective against the fully grown larvae of *Plodia interpunctella* Hb. and *Ephestia elutella* Hb. in the warehouse, and which is also suitable for general use on stored products.

Nothing is known about the problem of administering an atomised insecticide as a direct contact spray against caterpillars in warehouses. The optimum conditions of atomisation and air pressure and the best type of atomising nozzle to use have yet to be worked out.

It appears also that no technique and insecticidal material will be of general application. However, the material and methods described have been used in warehouses with success, and it is hoped that with some alterations they may be rendered suitable for other places.

Protection of operators.

When the atomised spray liquid is used in the manner described, particles of insecticide settle on all exposed parts of the operators. A certain amount of the material is liable to be inhaled, especially when using the method against moths.

The white oil carrier alone could have no ill effect either externally or when it is inhaled (20, p. 207). It may, however, have the secondary effect of keeping the dissolved pyrethrins and oleo-resins in contact with any tissue on which the insecticide settles.

The pyrethrins are generally considered to be non-toxic to warm-blooded animals. Zeigler(29) and Chevalier(2) state that intravenous infections of pyrethrins are capable of causing convulsions and death in dogs. Gnadinger(4), judging from his own experience and the work of Yamamoto, Zeigler and Chevalier, considers that the belief that the pyrethrins are non-toxic to warm-blooded animals is well founded.

It seems to be well established that pyrethrum powders and extracts can cause dermatitis. This affection is found in factories where the flowers are being ground, and according to Gnadinger(4) in rare instances when the acetone extract is being handled. It appears to be uncommon and only to occur in susceptible individuals. It does not seem to be quite clear how much of the effect is due to the pyrethrins and how much to the oleo-resins and other substances usually present with them.

During the hot summer of 1933 one of the operators working the guns developed a rash on the skin which was ascribed to the pyrethrum. None of the other operators working under the same conditions was in any way affected. It is stated by Kampmeier(9) that pyrethrum may cause hay fever and asthma. It was found that the atomised material

affected the mucous membrane and caused the eyes to smart and the nose to run. These symptoms did not develop seriously. One other effect was shown after about 3 months' continuous working without a mask. This consisted of slight indigestion which passed off as soon as the work was stopped for a day. Only two operators had worked for this length of time, and both reported the same symptoms.

After the case of skin rash, the precautions recommended by McCord, Kilker and Minster⁽¹⁵⁾ were adopted. The exposed parts of the operators were coated with cold cream before they started work and careful washing in alkaline water was enforced after the spraying was finished. Prosser-White⁽²¹⁾ gives a formula for a protective ointment to prevent any risk of this type of dermatitis, but it was not found necessary to use it.

In addition to the precautions recommended by McCord, Kilker and Minster (*loc. cit.*), the operators wore pads covering the mouth and nose which consisted of a square of cotton-wool cut from a roll of gamgee and held in place by a wire frame. This mask effectively prevented any of the atomised liquid from being inhaled. Goggles were provided at first to protect the eyes, but it was found that they were not necessary.

ASSESSMENT OF RESULTS OF LARGE-SCALE TESTS.

(a) *On moths.*

It is impossible accurately to assess quantitatively the effects of atomising a warehouse against moths, because it is not possible to ascertain the number of moths present before and after the process.

The method of using test insects is useless, because any form of container interferes with the distribution and effect of the insecticide.

There are two sources of information by which the effects of atomisation may be judged. The first is to examine a shed before and after each individual atomisation and note the condition of the moths present.

Some isolated observations of this nature were made in 1933 and 1934. In 1934 some localities were kept under observation over an extended period.

In 1933 the process was in the experimental stage and atomisation was not carried out every day during the period of moth emergence as was done in 1934. During the first year the sheds were inspected in the evening when the moths were most likely to be flying and those sheds noted where moths were present. These were treated, if possible, the next day.

The atomised sheds were examined, as a rule, on the following day for signs of dead moths. In some instances, where a detailed examination was thought desirable, a number of the moths brought down were collected

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and examined. The absence of any living moths the day after atomisation could not be used as a criterion of effectiveness, since emergence might have taken place after treatment.

An examination of the floor of the warehouse after atomisation did not, at first sight, show large numbers of dead moths. It was subsequently found that most of the moths brought down either crept under any convenient shelter or fluttered to the doors and crawled underneath. It was only by exposing such situations that the moths could be found.

During the inspections, preliminary to atomisation, in 1933 it was unusual to see more than ten moths, although in a few instances during the period of maximum emergence this number was exceeded. The most usual result was any number up to six. The following are some figures for dead moths found in the warehouses after atomisation:

Shed No. 1	55 moths
„ 2	220 „
„ 3	117 „
„ 4	78 „

These moths were collected from a small area of the ground in each shed, the number actually present over the whole area almost certainly amounting to several hundreds.

During 1934, when atomising was carried out every day, additional observations were made during the period from June 19th to 29th. Five collections were made from a number of situations in ten sheds and 173 *Ephestia elutella* Hb. and twenty-one *Plodia interpunctella* Hb. were found. During the 10 days over which this collection was made, never more than one live moth was seen in any of the ten sheds.

A collection was made every day from four localities over the period of 20 days from July 11th to 30th, 1934, with the following results:

Locality	<i>Plodia interpunctella</i> Hb.		<i>Ephestia elutella</i> Hb.		Total
	♂	♀	♂	♀	
Shed A	72	92	6	4	174
„ B	52	85	6	3	146
„ C	12	32	3	4	51
Between doors between two sheds	29	42	8	7	86

During the period over which these insects were collected no moths were seen during the day and only in isolated instances during the process of atomisation.

These collected observations point to two conclusions; first, that the number of living moths seen in an inspection of infested premises is very

much less than the number actually present; secondly, that the atomised insecticide will reach and kill a percentage of this latter number.

In 1933 some figures were obtained of the percentage of gravid females brought down by the process. The figures are quoted here:

	Few or no eggs laid	Eggs laid or partly laid	Percentage gravid
<i>Plodia interpunctella</i> Hb.	81	7	91.36
<i>Ephestia elutella</i> Hb.	103	17	83.50

The percentage of females that have laid their eggs will depend on the frequency with which the sheds are atomised. When the moths from which these figures were obtained were collected, the period between atomisations was 4 days. It is therefore evident that by shortening the interval between atomisations a kill of gravid females closely approximating to 100 per cent. could be obtained. An examination of the moths collected after atomisation showed that a large number of them had been affected before they had been able fully to expand their wings after emergence from the cocoon. The following figures were obtained:

Year	No. of moths examined	No. with wings not fully expanded	Percentage with wings not fully expanded
1933	78	40	51.3
1934	457	260	56.9

The theory put forward to account for the high percentage of moths with their wings not properly expanded is that a protective film of insecticidal material is formed over any exposed surfaces. Moths emerging from their cocoons and crawling on to any adjacent surface to stretch out their wings, come into contact with the film of insecticide and are affected.

The formation of a film is made possible by the non-volatile nature of the white oil carrying the insecticide, by the even coating resulting from atomising the liquid, and because the wood composing the cases of fruit and the roofs of the sheds does not absorb the material very rapidly. This is a valuable attribute of the atomisation process, because the film forms a protection against infestation during the periods between treatment. That this protective action may take place under other circumstances is shown by a statement made by T. J. Headlee in a discussion on a paper by Lathrop and Sazama (12). Headlee stated that an aqueous spray of pyrethrum and a white oil of viscosity 220 when applied to apple trees had given as good a protection as a lead arsenate spray when taken over a 10-day period.

The second source of information on the effect of the process is only available when atomisation is carried out every day during the period of moth emergence and where goods which are clean but liable to infes-

tation are stored in infested premises. Under these conditions the effectiveness of the process may be judged by the subsequent freedom of the goods from infestation.

These conditions were fulfilled in 1934 in two sheds containing dried fruit, and a comparison is drawn here between the infestation in 1933 when regular atomisation was not practised, and in 1934 when it was.

Shed No. 1 has a cubic capacity of about 149,000 cu. ft., and shed No. 2 a capacity of about 164,500 cu. ft. Each of these sheds contains, when full, between 35,000 and 38,000 cases of dried fruit, each case containing 56 lb. In both years under consideration the two sheds were full of fruit during the period of moth emergence.

In the beginning of the year 1933 the two sheds, then empty, were found to be badly infested with hibernating larvae which had migrated from the fruit during the previous year. They were therefore fumigated with hydrocyanic acid gas. By using test insects and also examining the sheds before and after fumigation, it was found that a high percentage of the insects present had been killed. A number of larvae survived in situations where the difficulties of penetration or the presence of a leak made it impossible to obtain a high concentration.

The two sheds were stocked with clean fruit before the end of May when moth emergence from the premises begins. The larvae that had survived the fumigation gave rise to moths which infested the clean fruit and necessitated the fumigation of 14,397 cases in shed No. 1 and 3796 cases in shed No. 2.

A considerable number of full-grown larvae crawled off the cases and reached the walls and floor. This was shown by a sticky band round the walls which caught a proportion of the migrating larvae. The sticky band, which had not been used in previous years, in addition to trapping some caterpillars, rendered the roofs inaccessible to the remainder. There were, however, a large number of places suitable as hibernating places for the larvae which were not isolated by the band. The empty sheds were not fumigated in 1934. The sheds were emptied of fruit between November 1933 and February 1934 and were restocked with clean fruit prior to the period of moth emergence in 1934.

Atomisation was carried out every day during the period of moth emergence in 1934. The presence of moths was noted during the process of atomisation on nine occasions in shed No. 1 and on five occasions in shed No. 2. Three inspections of stock for insect infestation were carried out between the beginning of June 1934 and the end of September 1934. No infestation was found in shed No. 1. One pile in a corner of shed

No. 2 was found to be infested sufficiently to necessitate the refumigation of ninety-five cases.

It is not possible to assess the exact significance of these figures, since the number of moths emerging from the premises in each year is not accurately known, but the available evidence indicates that there were at least as many moths emerging in 1934 as in 1933. In these circumstances the figures of 18,193 cases fumigated in 1933 as opposed to ninety-five in 1934 suggest that the routine atomisation practised was largely successful in preventing the moths emerging from the premises from reaching the goods stored in them.

(b) *On larvae.*

The full-grown larvae of *Ephestia elutella* Hb. and *Plodia interpunctella* Hb. in the warehouse have proved to be very resistant to the sprays and fumigants used against them, in comparison with most plant pests. It was also found that insects living under warehouse conditions were more resistant to fumigants than any bred so far in the laboratory, and it was thought that this might also be true of sprays.

In addition to the difference in resistance between the artificially bred insect and the insect in the warehouse, there appears to be a variation in resistance in the latter insect with the age of the fully fed larvae. Fully fed caterpillars at the beginning of the migratory phase appear to be more susceptible to sprays than they are towards the end of the migratory phase when they are preparing to hibernate. The factors influencing this change in susceptibility are not known. Physiological changes in the caterpillar and seasonal changes in temperature and moisture, which would affect both the metabolism of the insect and the physical and chemical properties of the spray, seem most likely to be important.

Because of the difference in susceptibility to the pyrethrum spray between warehouse-bred insects and those artificially bred, together with the possibility of seasonal changes in resistance, a short series of tests were made on the warehouse-bred insects under natural conditions.

An experiment under these conditions was thought to be the best starting-point in investigating the possibilities of the spray, since it would bring to light the difficulties that occur in practice but which may not occur in the laboratory. It would also serve as a guide to the strength of toxic agent and the amount of material required to kill the insects under natural conditions. The information thus gained should provide a basis for more accurate and detailed work in the laboratory with a

view to improving the spray material and the method of application.

Before giving an account of these tests it is necessary to make some observations on the conditions under which they were carried out.

The experiments were made rather late in the year. The main period of migration took place from about the end of the first week in August to about the middle of October in the dried fruit wharf. The length of this period is due to the unequal conditions of temperature and moisture in the sheds, as the life cycle proceeds more rapidly in the eaves and less rapidly as ground-level is approached. Some of the larvae used in the tests were obtained from another wharf where they had been feeding on cacao. This wharf consisted of several storeys, and the middle floors from which these larvae were collected had a more equable climate than the dried fruit sheds. The period of migration under these conditions ranged from about the third week in September to about the end of October. The spraying was done from November 16th to 24th, 1933, when the majority of the larvae in the warehouse had already spun their cocoons and the temperature was consequently low. The drop in temperature would affect both the metabolism of the insect and the qualities of the spray material.

The physiology of the effect of the pyrethrins on insects is not fully understood. Juillet⁽⁸⁾ considered that pyrethrum was a neuro-muscular poison; Slaing⁽²³⁾ concluded that it was a nerve poison; Kruger⁽¹⁰⁾ found that pyrethrum caused morphological changes in the hypodermis, muscles and nerve fibres of the larvae of *Corethra plumicornis*; Hartzell and Wilcoxon⁽⁷⁾ found that there were histological changes in the nerve ganglia of insects killed by pyrethrum. These last workers also found⁽⁶⁾ that a drop of concentrated pyrethrum extract caused death when applied to the tarsus of the rosechafer (*Macrodactylus subspinosus* Fab.) when that insect was so placed that the extract could not come into contact with any other part of the body. A contact poison which acts on either the nerve centres or the muscles is likely to have its effect reduced or inhibited by a slowing up of the insect metabolism, since the means of transport between one part of the body to the other, apart from physical diffusion, will be correspondingly reduced.

Two further effects of low temperature would be to decrease the chemical activity of the pyrethrins and increase the viscosity of the white oil carrier, both of which are likely to render the spray less effective. Moore⁽¹⁶⁾, when experimenting on the penetration of oils into the tracheae of the larvae of the wax moth, *Galleria melonella*, found that while a light

lubricating oil would penetrate, a heavy one would not. Viscosity and surface tension are the two factors affecting penetration, and since it would appear from the results of Green(5) that the surface tension of the different petroleum oils does not vary greatly, any increase in viscosity means a decrease in penetration, and hence a decrease in the toxic action of the spray. A study should be made of the effect of the spray at different temperatures with a view to finding out how it is affected by the range of temperatures likely to be met with in practice. The possibility of using heated jets in cold weather might also be studied.

Some evidence that caterpillars during the early migrating period in warmer weather were more susceptible than later in the year was gained from field observations. These observations are recorded together with the results of the spray tests.

SMALL-SCALE TESTS.

The following detailed tests were carried out under controlled conditions to obtain more definite assessment of the efficiency of the treatment.

The insects used were the fully grown larvae of *Plodia interpunctella* Hb. and *Ephestia elutella* Hb. collected from the wharf while migrating. They were collected as they were required for use as tests.

The larvae of *Plodia interpunctella* Hb. were all collected from the wharf in which the experiments were being conducted and had fed on dried grape fruits. Some of the larvae of *Ephestia elutella* Hb. were collected from the same wharf as the *Plodia interpunctella* Hb. where they had also fed on dried fruits, but others were collected from a wharf in which they had fed on raw cacao beans. The caterpillars that had fed on dried fruit were larger and more healthy looking than those that had fed on cacao, but the experiments did not show any difference in resistance between the two. It is quite possible, however, that the difference in food material had caused a difference in resistance, but that it was not sufficiently marked to be brought out by the tests. Some laboratory experiments might give some information on this question.

Apparatus used.

Plate XXXIII, fig. 3, is a photograph of the apparatus used in the experiment. The reservoir of one of the atomising guns, previously described, was removed, and the gun was then mounted on a platform (A) capable of horizontal swivelling and vertical adjustment.

The suction tube of the gun was connected by rubber tubing to a

graduated burette, and the air feed to the gun was connected through a pressure gauge (*B*) to the air compressor.

The gun and its mounting were set on an iron stand at a given distance from the target (*C*) on which the caterpillars to be sprayed were placed.

The target consisted of a wooden surface of 8 sq. ft. which was suspended vertically for spraying. The wooden surface was covered by a sheet of brown paper which was renewed for each experiment.

Procedure.

The pressure in the air compressor was fixed at 70 lb. per sq. in., since this was found by experiment to be the best in practice.

The burette was filled with the liquid to be used and the nozzle adjusted so that the degree of atomisation obtained was similar to that used in practice. This adjustment of the nozzle was then standardised by timing out a given quantity of the material.

The caterpillars to be sprayed were placed on a clean sheet of brown paper pinned to the target, the latter being raised so that its surface was horizontal. When the caterpillars had started to crawl the target was lowered to a vertical position and sprayed. It was found that with very few exceptions the caterpillars remained on the target during the spraying, those that fell off were not included in the tests.

Previously to spraying, the gun mounting had been set so that the target could be covered in two sweeps. The lower half of the target was covered with the first sweep; the gun was then shut off by releasing the trigger and the adjustment made for sweeping the top half of the target which was then sprayed. The interval between spraying the bottom half of the target and the top was very short, since the only adjustment necessary between the two sweeps was the removal of a block of wood.

Time did not permit of the addition of any mechanical device to perform the sweeping movement which, therefore, had to be done by hand. An even sweep was obtained by reading the level of the material in the burette at intervals of 0.5 c.c. The speed of the sweep was regulated by this means.

The dosage was administered in the following way. When the trigger of the gun was depressed the liquid that first sprayed out was absorbed by a pad of cotton-wool held immediately in front of the nozzle. When the working parts of the gun had become thoroughly coated with the liquid and a convenient level for reading had been reached in the burette, the cotton-wool pad was removed and the spraying commenced, when

8 c.c. had been sprayed out in the manner described, the trigger was released and the spraying ceased. At the end of each spraying, the apparatus was washed out by blowing through a quantity of ethylene trichloride and this was followed by a quantity of the liquid to be used in the next experiment.

After spraying the target was again raised to a horizontal position and the caterpillars were left for 10 min. to allow of a normal period of wetting.

They were then removed to glass-topped tins containing corrugated paper and placed in the warehouse under recorded conditions of temperature and humidity.

A complete account of an experiment of this nature should give the size of the atomised particles of the insecticide and the amount of the spray material adhering to the surface sprayed.

In the laboratory the quantity of material adhering to the sprayed surface can be obtained by weighing the spray platform before and after spraying, but in the experiment described this was not possible owing to the large size of the target.

It was evident that a considerable proportion of the material sprayed from the gun was prevented from reaching the target owing to air rebounding from its surface and by the eddy currents set up by the rapid air flow.

The problem of measuring the size of the atomised particles is difficult for two reasons. First because the nozzle used did not give a uniform degree of atomisation, and secondly because the size of any individual particle decreases in proportion to the length of time it has left the nozzle. The rate of decrease in size is governed by the volatility of the liquid composing the particle, the speed, size and initial temperature of the particle and the evaporating power of the air at the time.

In an experiment of this kind which was in the nature of a field trial it was not possible to take all these factors into account, so that the degree of atomisation was not measured but it was standardised for the type of nozzle and the material used.

The method of standardisation adopted in these tests was to record the time taken for a given quantity of material to be emitted from the nozzle under known conditions of temperature and air pressure and at a particular setting of the nozzle. It was found that with the nozzle set at the degree of atomisation required the time taken by a given quantity of material to blow through was fairly constant. Over eighteen readings the time taken for 8 c.c. varied between 31.0 and 34.5 sec.

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The following were the conditions of the experiment:

Quantity of material sprayed	8 c.c.
Size of target	8 sq. ft.
Therefore dosage	1 c.c. per sq. ft.
Distance from nozzle of gun to centre of target	4 ft. 6 in.
Air pressure before spraying	70 lb. per sq. in.
Air pressure during spraying	65 lb. per sq. in.
Therefore drop in pressure at the nozzle of the gun	5 lb. per sq. in.
Average time taken for 8 c.c. to blow through	33.6 sec.
Temperature range during spraying	46-50° F.
Humidity range during spraying	70-82 per cent. R.H.
Period during which the insects were sprayed	Nov. 16th to 24th, 1933

Tables II and III show the results of spraying.

Table II.

Plodia interpunctella Hb.

Spray No.	Strength pyrethrins I and II	No. sprayed	Moribund and dead	Live	Moribund	Percentage kill moribund and dead
1	Pure white oil	85	7	78	0	8.2
2	0.33	109	40	69	0	36.7
3	0.41	94	73	21	1	77.7
4	0.54	92	91	1	5	98.9
5	0.81	83	83	0	0	100.0
6	1.63	93	91	2	9	97.8
7	Controls	155	2	153	—	1.3

Table III.

Ephestia elutella Hb.

Spray No.	Strength pyrethrins I and II	No. sprayed	Moribund and dead	Live	Moribund	Percentage kill moribund and dead
1	Pure white oil	85	20	65	6	23.5
2	0.33	97	93	4	1	95.7
3	0.41	92	90	2	1	97.8
4	0.54	96	95	1	4	99.0
5	0.81	94	94	0	3	100.0
6	1.63	80	80	0	0	100.0
7	Controls	94	0	94	0	0.0

DISCUSSION OF RESULTS.

From the results of these tests it appears that the larvae of *Ephestia elutella* Hb. are considerably less resistant than those of *Plodia interpunctella* Hb. the latter insects proving extremely resistant under the conditions of the experiment. A strength of 0.81 per cent. pyrethrins

Table IV.

Showing the temperature and humidity conditions in the warehouse under which the test insects were kept after spraying.

Week beginning	Max. temperature °F.	Min. temperature °F.	Max. R.H. %	Min. R.H. %
Nov. 13th, 1933	48	46	88	62
„ 20th, 1933	50	43	88	64
„ 27th, 1933	44	38	76	60
Dec. 4th, 1933	42	38	79	55
„ 11th, 1933	40	35	72	45
„ 18th, 1933	41	38	73	62
„ 25th, 1933	40	36	86	63
Jan. 1st, 1934	43	35	86	68
„ 8th, 1934	44	42	80	70

I and II in the white oil specified should kill the migratory larvae of *Ephestia elutella* under any climatic conditions normally occurring in the London warehouses, provided that the dosage and its method of application are similar to that described.

The position with regard to *Plodia interpunctella* is not so clear. Over a range of 0.54–1.63 per cent. pyrethrins I and II a good kill is secured, but there is no margin of safety. It would be impractical to use any higher percentage of pyrethrins since the cost would be prohibitive. Further work should be done with a view to increasing the kill by other means. Three lines of investigation are possible; the first is to try the effect of an increased dosage with a lower pyrethrin content, the second is to use another or an additional toxic agent, and the third is to alter the physical properties of the carrier. The first of these estimations appears, from practical experience, to promise good results but no quantitative data is as yet available.

Some evidence that caterpillars during the main migratory period in warmer weather are more susceptible than later in the year was gained from field observations in the warehouse. Some sheds were atomised against moths during the early period of migration of the larvae. After the process a considerable number of larvae were found to be affected with the typical symptoms of pyrethrum poisoning. Fifty-one larvae were collected and of these twenty-one died. These insects were only exposed to a mist, so that a kill of 42 per cent. makes a good comparison with the results obtained in the tests where the insects were sprayed directly.

On three other occasions caterpillars that had been directly sprayed in the warehouse with a solution of 1.63 per cent. total pyrethrins in white oil were collected and kept. Of these insects 110 were *Plodia*

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interpunctella Hb. and two were *Ephestia elutella* Hb.; all were killed. The insects in this instance were taken at random from sheds where spraying against larvae was in progress and they were thus subjected to the conditions that obtain in practice.

As there is no accurate data on the dosage applied to the insects in the sheds it is not possible to compare the results closely with those obtained in the tests. It is, however, unlikely that the insects in the sheds were given a significantly larger dose than that administered in the tests.

The inhibiting effect of cold weather on the activity of the pyrethrins against insects has also been noted by Fleming(3). This author, in discussing the use of aqueous pyrethrum sprays, states that Japanese beetles are more susceptible in warm weather (80° F.) and that it is almost futile to use the spray under adverse weather conditions.

Apart from the fact that 100 per cent. kill was obtained in the three instances when larvae were collected after spraying during the warmer weather, the results were interesting because the larvae all died within a few days of spraying. Some showed no signs of life after a few hours, thus contrasting strongly with those sprayed in the tests in which the majority of the larvae lingered on for several weeks in a moribund condition.

Hartzell and Wilcoxon(7), when experimenting on the action of the pyrethrins on the adult rosechafer (*Macrodactylus subspinosus* Fab.), found that both death and the process of recovery are accelerated by an increase of temperature.

It would therefore appear that during the higher temperatures prevailing coincident with the main migration period, the spray was more effective than was shown by the tests. At this stage it is not possible to say how much of this increased effectiveness is due to the increased activity of the spray and how much is due to the more rapid metabolism of the insect.

Ten minutes or more elapsed after spraying before the majority of the larvae in the tests showed signs of pyrethrum poisoning and the symptoms were not as violent as those shown earlier in the year.

Apart from those sprayed with pure white oil, none of the treated larvae made any attempt to open cocoons, those that survived merely crawled into a suitable place and attached themselves by a few threads to the support.

As the position stands at present, a material and mode of application have been worked out for the first time which will give a high per-

centage, if not a complete kill of the larvae of *Ephestia elutella* Hb. and *Plodia interpunctella* Hb. and which is suitable for use on stored edible products and other goods in warehouses. Both the material and methods could be improved, and in order to do this a considerable amount of experimental work must be done in the laboratory. Some of the technical problems brought out by this work are now being studied by Mr Scott of the Imperial College Biological Field Station, in connection with work on the comparison of the toxicity of insecticides.

SUMMARY.

(a) The problem of the infestation of imported stored products by the Phycitid moths, *Ephestia elutella* Hb. and *Plodia interpunctella* Hb., is outlined.

(b) An outline account is given of the life history of *Ephestia elutella* Hb. and *Plodia interpunctella* Hb. in the London warehouse.

(c) The specification of the insecticidal material used is given, together with a description of its properties and the factors influencing its choice.

(d) An account is given of inflammability tests on the material when in an atomised state.

(e) The apparatus used is described.

(f) The process as used against moths is described.

(g) The process as used against migrating larvae is described.

(h) The precautions which should be taken by operators are given.

(i) An account is given of a series of biological tests and observations made on the effects of this material and process on the moths and larvae of *Plodia interpunctella* Hb. and *Ephestia elutella* Hb.

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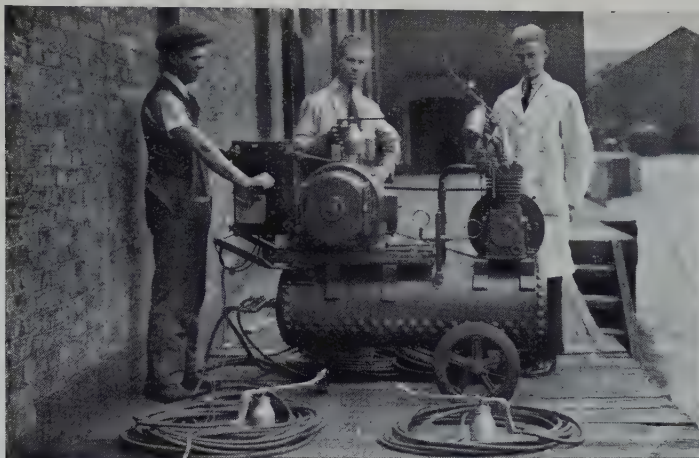


Fig. 1.

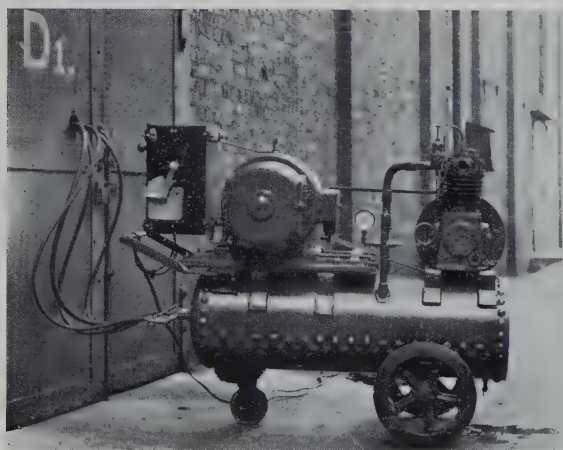


Fig. 2.

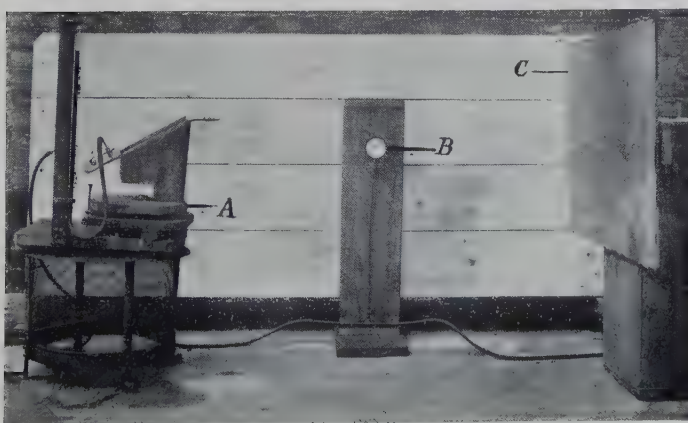


Fig. 3.

POTTER.—AN ACCOUNT OF THE CONSTITUTION AND USE OF AN ATOMISED WHITE OIL—PYRETHRUM FLUID—TO CONTROL *Plodia interpunctella* HB. AND *Ephestia elutella* HB. IN WAREHOUSES (pp. 769–805).

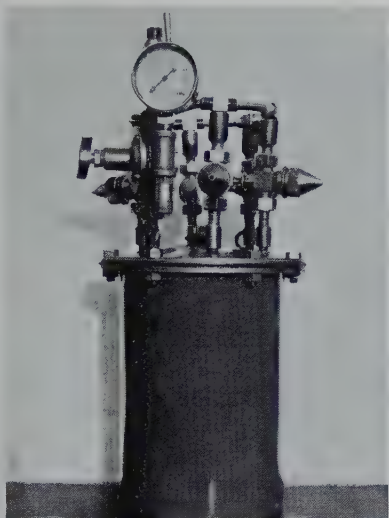


Fig. 1.

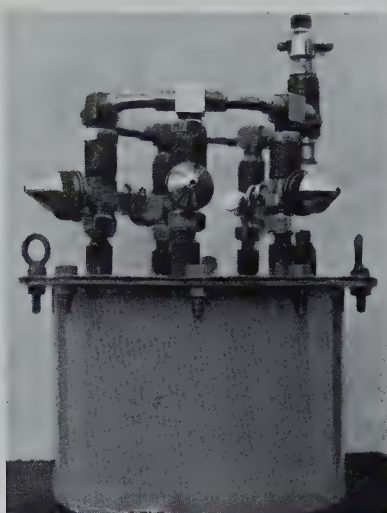


Fig. 2.

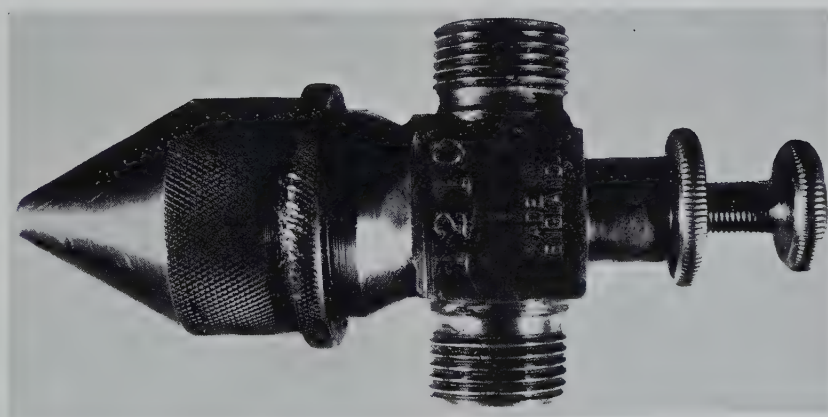


Fig. 3.

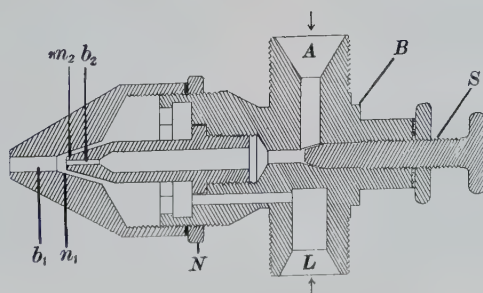


Fig. 4.

POTTER.—AN ACCOUNT OF THE CONSTITUTION AND USE OF AN ATOMISED WHITE OIL—PYRETHRUM FLUID—TO CONTROL *PLODIA INTERPUNCTELLA* HB. AND *EPHESTIA ELUTELLA* HB. IN WAREHOUSES (pp. 769–805).

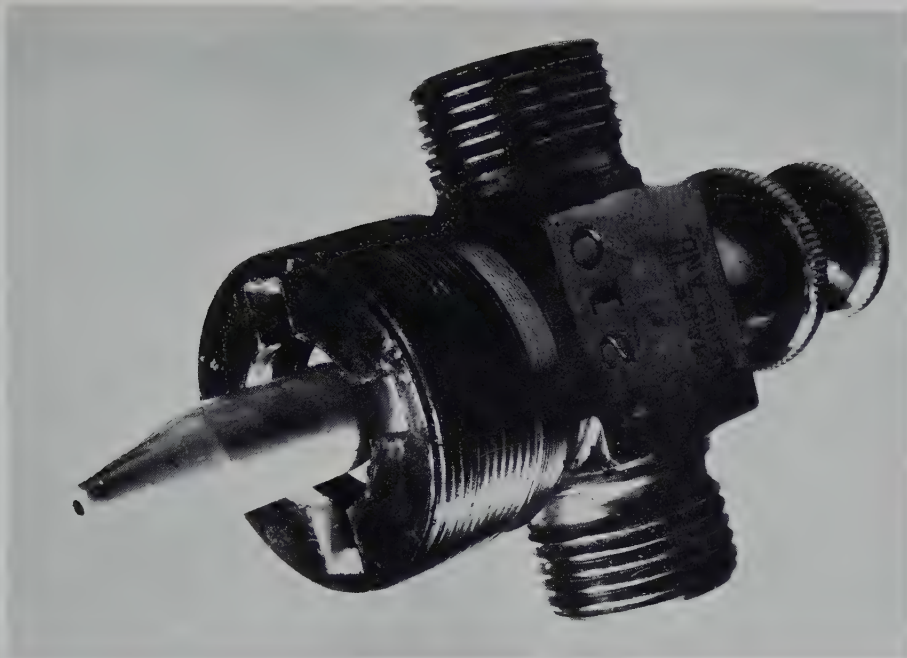


Fig. 1.

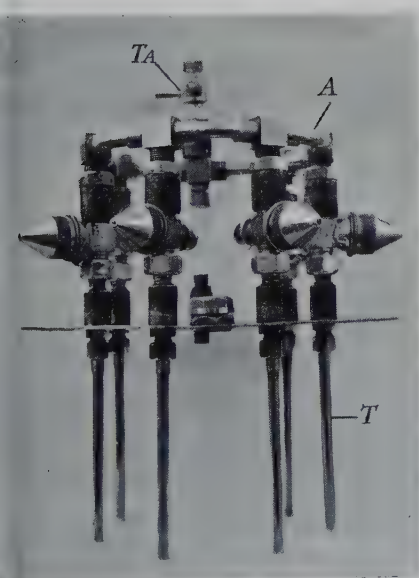


Fig. 2.

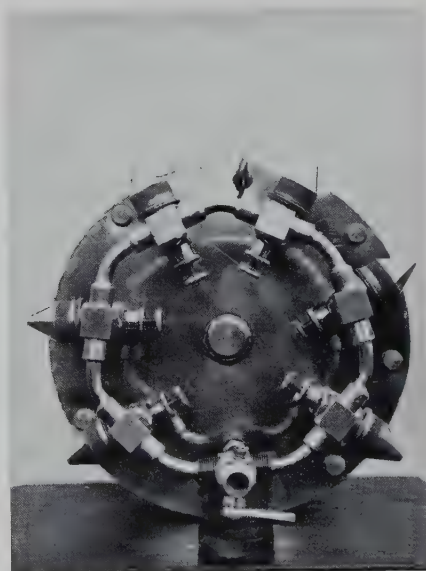


Fig. 3.

OTTER.—AN ACCOUNT OF THE CONSTITUTION AND USE OF AN ATOMISED WHITE OIL—PYRETHRUM
 LUID—TO CONTROL *PLODIA INTERPUNCTELLA* HB. AND *EPHESTIA ELUTELLA* HB. IN WAREHOUSES
 (pp. 769-805).

EXPLANATION OF PLATES XXXIII—XXXV.

PLATE XXXIII.

- Fig. 1. Complete atomising apparatus consisting of an air compressor and four guns.
Fig. 2. The air compressor in working position with the hoses leading into the shed to convey the compressed air to the atomising apparatus.
Fig. 3. Apparatus used for small-scale tests against larvae of *Plodia interpunctella* and *Ephestia elutella*. A=Atomising gun mounted on adjustable platform. B=Pressure guage. C=Target.

PLATE XXXIV.

- Fig. 1. Experimental atomising unit.
Fig. 2. Final design of atomising unit.
Fig. 3. Nozzle used in atomising unit.
Fig. 4. Transverse section through a nozzle used in atomising unit.

PLATE XXXV.

- Fig. 1. Nozzle used in atomising unit, with outer cone and lock-nut removed.
Fig. 2. Atomising unit with reservoir removed. A=Air ring. T_A=Compressed air tap.
T=Liquid feed tube.
Fig. 3. Top of atomising unit showing disposition of nozzles round the air ring.

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REVIEWS

Chronica Botanica. Vol. I. Edited by FR. VERDOORN. Pp. 447. P.O. Box 8, Leiden, Holland. 1935. 15 Dutch Guilders.

In the "blurb" to *Chronica Botanica* one reads: "There are nearly 4000 institutions of pure and applied botany. There are between 60,000 and 70,000 botanists. There are about 1000 periodicals concerned with botany! How can you keep in touch with all this activity? How can you find out what other botanists are doing and what new work they are planning?" It is comforting to read that "*Chronica Botanica* will help you" and that "*Chronica Botanica* will answer hundreds of questions, previously left unanswered, although they often were of fundamental importance for the continued progress of your work".

The volume opens with an Almanac, January 1935–April 1936, containing a few dates of conferences and of birthdays of various botanists who entered life prior to 1886. This is followed by the programme of the Sixth International Congress to be held in Amsterdam in September 1935 with photographs of its committee and recorders, photographs of twelve eminent botanists who died during the period 1930–33, and a brief history of International Congresses contributed by A. B. Rendle. There are then 35 pages devoted to various important congresses, committees and societies. The main part of the volume, some 260 pages in English, French, German, Italian and Spanish, contains a "Review of all Branches of Plant Science during 1934" arranged alphabetically by countries and towns and illustrated by numerous photographs of botanists, buildings, gardens, expeditions, etc. There follow ten pages of "Correspondence and Queries", two pages announcing the birth of 24 "New Periodicals", 33 pages of "New and Changed Addresses" arranged alphabetically under countries, a page of "Editorial Notes", four pages of "A short Illustrated History of the Botany of the Netherlands through the eyes of J. Lanjouw and H. Uittien and drawn for Members of the Sixth International Botanical Congress by H. Ramaer Jr.", 45 pages of advertisements, an "Index of Plant Names and Plant Parasites" (about 1250), and an "Index of Persons" (about 6000).

The Almanac is just an almanac; the data regarding congresses and societies may be found useful; the address list will be found useful; the comic strip of Dutch botanical history is boring. The "blurb" states that "*Chronica Botanica* brings you each year from the whole world a review of the important Current Research in all branches of Plant Science", and that this "is considered the chief feature of the new year book". This, of course, is a joke, but one can spend a pleasant hour browsing through these pages learning the personal and scientific news of all sorts of institutions one has never heard of. At first one is inclined to ridicule these pages which are often unbalanced and not infrequently merely pander to personal, departmental and institutional prestige. The editor assures us that "In publishing or refraining from publishing any notes, photographs etc., in publishing in the original form or in an abridged form any material submitted, we are guided by well considered arguments. We wish to emphasize that the length of an item is no criterion with which to judge the scientific importance of the matter contained therein". Still it does seem a little unbalanced and lacking in perspective that, in Great Britain for example, the Long Ashton Research Station, the Wye Agricultural College and the botany departments of the universities of Birmingham, Reading, Sheffield, of Bedford College and of Nottingham University College should be cited by name only whereas the Alpine Garden Society is allotted a whole column and Flora's League two-thirds of a column. Or, to take American examples, the botany departments of John Hopkins University, Harvard, Pennsylvania, Wisconsin, Minnesota, Ohio, Kansas, and Maine are cited by name only, whereas the N.Y. Botanic Gardens receives five columns, the San Diego Society of Natural History half a column and even the Department of Biology of Juniata

College (described as "Denominational Coeducational College, Protestant, Church of the Brethren, otherwise called 'Dunkards'") some eleven lines. Also one wonders whether much of the "news" is really of the slightest botanical interest, e.g. of the late Mrs X that "Her husband is the Professor of Chemistry in this College and she leaves three children", or of Dr Y that "while he had been retired from active service on its staff for several years his support and counsel are much missed", that Harvard "Forest as a seller of lumber has been operating under the provisions of the Code of Fair Competition for the Lumber and Timber Products Industries since it came into effect in August 1933", or that of the U.S. Botanic Garden "Our material wealth and resources are unbounded. Our latent cultural and spiritual wealth is equally unbounded if we grasp the opportunity offered to us. And it is a source of great satisfaction to know that our people as represented in our Federal Government, have never evaded a responsibility when that responsibility was clearly set out....At best is it not reasonable to assume that our right hand should know what our left hand is doing?" And there are quantities of this sort of stuff. Leaving trivialities on one side, however, one is impressed by the number of interesting data these pages contain. From them one really can, if one takes the trouble, obtain a fair idea of botanical happenings during the last year or two and, with the changes which the editor foreshadows in his notes, the value and interest of this section should be greatly enhanced in future volumes.

The idea of *Chronica Botanica* is probably sound, and if all botanical institutions and societies would make adequate annual returns the volume could become of considerable usefulness and historical value. Even as a preliminary experiment it has partially succeeded and, with more restrained and judicious recording in the questionnaire, and much greater editorial severity and discrimination, the future of the venture should be assured. The most obvious factors militating against success are the slackness of botanists in keeping the editor up-to-date and the price of the volume which, in depreciated exchange values, is excessive.

WILLIAM B. BRIERLEY.

SORAUER, P. *Handbuch der Pflanzenkrankheiten*. Herausgegeben von Dr O. APPEL. 6th ed. Bd. I. *Die nichtparasitären- und Virus-Krankheiten*. Teil 2. Pp. viii+553. Berlin: Paul Parey. 1934. 44 gold marks.

PART I of vol. I of the sixth edition of Sorauer's *Handbuch* was published late in 1933 and was reviewed in this *Journal*, XXI, 3, August 1934. The second part, now issued, completes the volume devoted to non-parasitic and virus diseases of plants. Part I contained historical and general introductions, and the first three chapters of the special portion dealing respectively with plant nutrition in relation to plant disease, climatic factors as causes of disease, and the effects of extremes of temperature. Part II has independent pagination but continues the special portion, commencing with chap. IV.

Chap. IV, 79 pages by Prof. K. O. Müller, is devoted to a genetical consideration of plant diseases determined by internal causes; dwarfing, atrophy and loss of organs, chlorophyll defects, necrosis, failure of flower development, premature dropping of flowers, and abortion of the sexual apparatus. Chap. V, 86 pages by Dr E. Pfeil, contains an account of plant diseases brought about by unfavourable physical and chemical conditions such as abnormal soil structure, unsuitable water, air and temperature relations, excessive acidity and alkalinity, the accumulation of harmful substances, and excess or deficiency of boron, magnesium and other elements. It is an excellent summary of these diffuse problems and contains a useful discussion of soil sickness. Chap. VI, by Dr O. Schlumberger, is devoted to wounds and its 55 pages are arranged in four sections dealing respectively with the causes of wounds, the plant's reaction, the regeneration of cells and tissues and, all too briefly, the effects of wounding on the general development of plants. This chapter is one of the best short

accounts I know of the subject. Chap. VII, 67 pages by Prof. E. Tiegs, discusses plant injury caused by smoke and fumes containing such products as various acids, the halogens, ammonia, illuminating gas, etc. Chap. VIII, 29 pages by the same author, is devoted to plant injury caused by industrial effluents and sewage, important recent problems of which little is definitely known.

All the above chapters deal with difficult and obscure subjects of which our knowledge is mostly very unsatisfactory. The causes, symptoms, host reactions and prevention of such diseased conditions are usually vague and diffuse and their elucidation difficult. They are a "no-man's-land" avoided alike by physiologist and pathologist and usually abandoned to the chemist, and yet they are among the problems whose botanical study would most repay investigation and whose practical solution would most ameliorate crop culture. Our attention, following a line of least resistance, has been over restricted to parasitic diseases and a greater study of physiological pathology would lead to a major advance in our understanding of the fundamentals of health and disease in plants. The clear and authoritative statement of the problems, and the accurate and precise way in which our present knowledge, such as it is, has been summarised in these chapters are wholly admirable and pathologists owe a debt of gratitude to the several authors.

Chap. IX, which occupies the remaining 182 pages of the book, is by Dr E. Köhler and is quite distinct from the foregoing, being devoted to virus diseases. Its presence here reflects the tendency still marked in many continental botanists and pathologists to regard virus diseases as more nearly related to non-parasitic than to parasitic diseases. Following a brief introduction, there is a general section of 57 pages devoted to etiology, symptomatology, the development of disease in the plant and the relation of external factors, host recovery, disease transmission, manifestations of resistance, study *in vitro*, classification of viruses, and disease control. There follows a special section of 122 pages of which 63 are devoted to virus diseases of the Solanaceae and the remainder to virus diseases of other plants arranged by families. This chapter will be read with interest by workers in England and America, for it is the first comprehensive statement on plant virus diseases to emanate from Germany and many of the points of view do not entirely coincide with those of workers in other countries. The chapter is well balanced and excellently put together, although the illustrations are inadequate, especially that depicting intracellular bodies.

The book opens with a detailed table of contents, which is useful, and closes with a very good index which serves both parts of vol. I. There are 147 text-figures, some of which are original, but it is a great pity that the work is not more fully illustrated. The numerous references include publications of the year 1933, and are inserted as footnotes. The proof correcting of the citations has been somewhat casual and there is no author index. The book is beautifully printed and produced. Part II confirms the high standard set by the previous volume and one must congratulate its distinguished editor, Dr O. Appel, and his collaborators, all of Berlin-Dahlem, on a noteworthy achievement. It is very unfortunate that in these days of depreciated exchanges the cost of the book will place it beyond the reach of many who would find it useful. There is no work in the English language which even attempts to cover the field of non-parasitic diseases as it is considered in vol. I of Sorauer's *Handbuch* and it is greatly to be hoped that arrangements will be made for an English translation.

WILLIAM B. BRIERLEY.

Succulent Plants: description, cultivation and use of succulent plants, other than Cacti. By H. JACOBSEN. Authorised translation by VERA HIGGINS. Pp. xvi + 293, figs. 277. London: Williams and Norgate. 1935. Price 25s.

This book is a translation of *Die Sukkulanten* (Paul Parey, Berlin, 1923), but much additional matter has been incorporated, together with many new illustrations.

The need has long been felt for a comprehensive and authoritative work in English on this group of plants. Together with the Cacti, the other succulents are rapidly

gaining in importance in the horticultural world. After a certain popularity during the middle years of last century, interest in these plants waned, but in late years there has been a recrudescence of interest to such an extent that there are in existence now several firms specializing almost exclusively in the growing of succulents.

The Cactus and Succulent Society of Great Britain has a large and increasing membership and holds an annual two-day Show in the Royal Horticultural Society's Hall. It was fitting that the publication of the translation of Jacobsen's standard work by Mrs Higgins, the Secretary of the Society, should coincide with this year's Show.

The book contains five chapters, of which the last comprises the main part. Chap. I deals briefly but adequately with the geographical and ecological distribution of the succulents. Chap. II gives an account of the structure and form of these plants. In a group containing such a wide diversity of structure it is difficult to deal in any way adequately with the subject and the original author was wise in confining himself to three pages, giving very briefly the principal modifications which occur in the types. Chap. III, on uses and cultivation, is rather disappointing. The directions for cultivation, supplemented by the additional remarks on the special requirements of certain genera contained in the main body of the text, are perhaps sufficient, but much more might with advantage have been written on the propagation of this physiologically interesting group. Propagation by cuttings is dismissed in two sentences, although the special needs of some forms are of unusual interest. Some of these special methods are dealt with, it is true, under the cultivation of *Mesembrianthemums*, but the requirements of other genera are not mentioned. The word "Uses" in the title of Chap. III would have been more appropriate for Chap. IV, which gives lists of the uses for which these plants are grown in nursery practice. Chap. V, which constitutes the main part of the work, comprises a list of the genera arranged in alphabetical order, with the principal species in each genus.

No attempt has been made to give a full botanical description of each species, but the notes given are sufficient in most instances for identification.

Much rearrangement and renaming has occurred in this group in recent years and the author has greatly facilitated reference to any particular species and the determination of the valid name by very adequate cross-referencing of synonyms. The new genera into which the *Mesembrianthema* have been divided are, for ease of reference, arranged alphabetically under the one heading "*Mesembrianthemum*".

The illustrations, all photographic, are excellent and are aided in clarity by the high quality of the paper.

This book, together with Brown, Tischer and Karsten's *Mesembryanthema* and the recent works of Mrs Higgins and of Houghton on Cacti, will rank as a standard work on this interesting group for many years.

R. H. STOUGHTON.

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- ESCHERICH, Prof. Dr KARL, Institut für Angewandte Zoologie, München, N.W. 2, Germany.
- GÄUMANN, Prof. ERNST., Eidgenössische Technische Hochschule, Universitätstrasse, Zurich, Switzerland.
- HOPKINS, Dr A. D., Bureau of Entomology, Department of Agriculture, Washington, D.C., U.S.A.
- HOWARD, Dr L. O., late Principal Entomologist, Department of Agriculture, Washington, D.C., U.S.A.
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- VAVILOV, Dr N. I., Director, Institute of Plant Industry, Rue Herzen 44, Leningrad, U.S.S.R.

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- 1931 AINSWORTH, G. C., Ph.D., Ph.C., Experimental and Research Station, Cheshunt, Herts.
- 1920 ALCOCK, Mrs N. L., M.B.E., F.L.S., Royal Botanic Gardens, Inverleith Row, Edinburgh.
- 1928 ALLEN, L. A., M.Sc., Ph.D., The University, Reading, Berks.
- 1908 ASHWORTH, Prof. J. H., D.Sc., F.R.S., Department of Zoology, University of Edinburgh, West Mains Road, Edinburgh. (Council, 1923-1925.)
- 1914 BAILEY, M. A., M.C., M.A., Controller of Agricultural Research, Gezira Agricultural Research Service, Shambat, Sudan.
- 1926 BALFOUR-BROWNE, Prof. F., M.A., Winscombe Court, Winscombe, Somerset.
- 1914 BARKER, Prof. B. T. P., M.A., Director, Research Station, Long Ashton, Bristol. (Council, 1916-1920, 1926-1928.)
- 1931 BARNARD, Mrs DOROTHEA E., c/o B.O.C., No. 102 Bungalow, Khodaung, Upper Burma.

- 1927 BARNES, H. F., M.A., Ph.D., Rothamsted Experimental Station, Harpenden, Herts.
- 1922 BARRATT, Miss K., D.Sc., Principal, Horticultural College, Swanley, Kent.
- 1929 BARRITT, N. W., M.A., Rothamsted Experimental Station, Harpenden, Herts.
- 1931 BARTON-WRIGHT, E., M.Sc., Flour Millers' Research Laboratory, Old London Road, St Albans, Herts.
- 1930 BAWDEN, F. C., B.A., Potato Virus Research Institute, School of Agriculture, Cambridge.
- 1923 BAXTER, D. EYRE, Kuala Padah Estate, Bentong, Pahang, Federated Malay States.
- 1932 BEAUMONT, A., M.A., Seale-Hayne Agricultural College, Newton Abbot, Devon.
- 1928 BENNETT, F. T., B.Sc., Ph.D., N.D.A., Agricultural Department, Armstrong College, Newcastle-upon-Tyne.
- 1920 BERRIDGE, Miss E. M., D.Sc., F.L.S., Witheridge, Lebanon Gardens, Biggin Hill, Kent.
- 1919 BEWLEY, W. F., C.B.E., D.Sc., Director, Experimental and Research Station, Cheshunt, Herts. (Council, 1922-1924, 1929.)
- 1927 BISSETT, N., M.R.C.V.S., University College, Newport Road, Cardiff.
- 1920 BLACKMAN, Prof. F. F., M.A., Sc.D., F.R.S., St John's College, Cambridge.
- 1932 BLACKMAN, G. E., B.A., Jealotts Hill Agricultural Research Station, Warfield, Bracknell, Berks.
- 1919 BLACKMAN, Prof. V. H., M.A., Sc.D., F.R.S., Imperial College of Science, London, S.W. 7. (President, 1924-1925; Vice-President, 1922-1923; Council, 1920-1921, 1926-1931.)
- 1920 BORTHWICK, Prof. A. W., O.B.E., D.Sc., School of Forestry, University of Aberdeen. (Council, 1925-1926.)
- 1935 BOYD, D. O., B.Sc., Entomology Dept., The University, Reading.
- 1919 BRENCHLEY, Miss W. E., D.Sc., F.L.S., F.R.E.S., Rothamsted Experimental Station, Harpenden, Herts.
- 1914 BRIERLEY, Prof. W. B., D.Sc., F.L.S., Dept. of Agricultural Botany, University of Reading, Berks. (President, 1932-1933; Vice-President, 1930-1931; General and Botanical Secretary, 1920-1922; General and Botanical Editor, 1921- .)
- 1914 BROOKS, F. T., M.A., F.R.S., F.L.S., The Botany School, Cambridge. (Vice-President, 1928-1929; Council, 1921-1922, 1927-1930.)
- 1921 BROOKS, R. ST-JOHN, M.D., M.A., D.P.H., D.T.M. and H., Lister Institute, Chelsea Bridge Road, London, S.W. 1.
- 1933 BROWN, J. M. B., B.Sc., Imperial Forestry Institute, Oxford.
- 1931 BROWN, R., B.Sc., Seale-Hayne Agricultural College, Newton Abbot, Devon.
- 1924 BROWN, Prof. W., M.A., D.Sc., Imperial College of Science, London, S.W. 7. (Vice-President, 1933, 1935; Botanical Secretary, 1928-1932; Council, 1933-1935.)
- 1924 BUCKHURST, A. S., O.B.E., A.R.C.S., D.I.C., Pathological Laboratory, Milton Road, Harpenden, Herts. (Council, 1935- .)
- 1920 BUDDIN, W., M.A., The University, Reading, Berks. (Council, 1932-1934.)
- 1928 BURR, S., M.Sc., Assistant Lecturer, Department of Agriculture, The University, Leeds.

- 1928 BUSHBY, L. C., F.R.E.S., F.Z.S., Curator of Insects, Zoological Society of London, Regent's Park, London, N.W. 8.
- 1920 BUTLER, E. J., C.M.G., C.I.E., D.Sc., M.B., F.R.S., F.L.S., Secretary, Agricultural Research Council, 5 Dean's Yard, Westminster, S.W. 1. (President, 1928-1929; Vice-President, 1924-1925; Council, 1921-1925, 1930-1931.)
- 1930 CALDWELL, J., D.Sc., Ph.D., Dept. of Botany, University College, Exeter.
- 1934 CAMERON, A. E., M.A., D.Sc., F.R.E.S., Dept. of Agricultural Zoology, The University, Edinburgh.
- 1932 CAMPBELL, A. H., Ph.D., The University, Bristol.
- 1928 CANN, F. R., D.I.C., Assistant Entomologist, Forest Products Research Laboratory, Princes Risborough, Bucks.
- 1935 CARLETON, Miss M., B.Sc., Dept. of Zoology, The University, Bristol.
- 1927 CARROLL, J., M.Sc., D.I.C., A.R.C.S., Albert Agricultural College, Glasnevin, Dublin, Irish Free State.
- 1929 CARTWRIGHT, K. T. ST GEORGE, M.A., Forest Products Research Laboratory, Princes Risborough, Bucks. (Council, 1931-1933.)
- 1914 CAYLEY, Miss D. M., D.Sc., John Innes Horticultural Institute, Merton, Surrey, S.W. 19. (Council, 1925-1926.)
- 1905 CHANDLER, S. E., D.Sc., F.L.S., Imperial Institute, London, S.W. 7. (Secretary, 1912-1913; Council, 1914-1920.)
- 1925 CHEAL, W. F., Gosmoor Lane, Elm, Nr. Wisbech.
- 1926 CHEESMAN, Prof. E. E., B.Sc., A.R.C.S., Imperial College of Tropical Agriculture, Trinidad.
- 1930 CHESTERS, C. G. C., M.Sc., Department of Botany, The University, Edgbaston, Birmingham.
- 1931 CHIPPINDALE, H. G., M.Sc., University College of Wales, Aberystwyth.
- 1908 CHITTENDEN, F. J., F.L.S., V.M.H., Royal Horticultural Society, Vincent Square, London, S.W. 1. (Council, 1914-1921.)
- 1921 CHRYSTAL, R. N., D.Sc., Imperial Forestry Institute, Oxford. (Council, 1931-1933.)
- 1905 CORNWALLIS*, F. S. W., Linton Park, Maidstone, Kent.
- 1915 COTTON, A. D., F.L.S., Royal Botanic Gardens, Kew, Surrey. (Vice-President, 1932, 1934; Council, 1917-1921, 1923-1925, 1932-1934.)
- 1931 CRAIGIE, J. H., A.B., M.Sc., Ph.D., Dominion Rust Research Laboratory, Winnipeg, Manitoba, Canada.
- 1920 CUNLIFFE, N., M.A., D.Sc., School of Rural Economy, University, Oxford. (Council, 1927-1929.)
- 1929 CURTIS, Miss K. M., M.A., D.Sc., F.L.S., Mycologist, Cawthron Institute, Nelson, New Zealand.
- 1920 CUTLER, D. WARD, M.A., F.L.S., Rothamsted Experimental Station, Harpenden, Herts. (Zoological Editor, 1921-1932.)
- 1927 DADE, H. A., A.R.C.S., Mycologist, Research Branch, Department of Agriculture, Aburi, Gold Coast, W. Africa.
- 1915 DAVIDSON, J., D.Sc., F.L.S., F.R.E.S., Waite Agricultural Research Institute, University of Adelaide, Glen Osmond, S. Australia.
- 1927 DAVIES, W. MALDWYN, Ph.D., University College, Memorial Buildings, Bangor, N. Wales. (Council, 1935- .)

- 1930 DAWSON, R. B., M.Sc., F.L.S., Director, St Ives Research Station, Bingley, Yorkshire.
- 1932 DINELEY, Mrs D., M.Sc., 5 Drayton Gardens, London, S.W. 10.
- 1923 DIXON*, Miss A., M.Sc., F.R.M.S., Rothamsted Experimental Station, Harpenden, Herts.
- 1930 DONCASTER, J. P., B.A.; School of Agriculture, Cambridge.
- 1920 DOWSON, W. J., M.A., D.Sc., Botany School, Cambridge.
- 1920 DRUMMOND, Prof. J. M., M.A., F.L.S., Botany School, The University, Manchester.
- 1935 DUFFIELD, C. A. W., F.R.E.S., The Cooper Technical Bureau, 47, Russell Square, London, W.C. 1.
- 1923 DU PORTE, ERNEST MELVILLE, M.Sc., Ph.D., F.R.E.S., F.R.M.S., Macdonald College, Montreal, Canada.
- 1928 EASTHAM, Prof. L. E. S., M.A., M.Sc., Department of Zoology, The University, Sheffield.
- 1927 EDWARDS, E. E., M.Sc., Department of Agricultural Zoology, University College, Cardiff.
- 1935 EKINS, Miss E. H., B.Sc., Principal, The College, Studley, Warwickshire.
- 1919 ELLIOTT, Mrs J. S. BAYLISS, D.Sc., Botany School, The University, Birmingham.
- 1934 ENGLEDDOW, Prof. F. L., M.A., C.M.G., School of Agriculture, Cambridge.
- 1922 ESDAILE, Miss P. C., D.Sc., F.Z.S., King's College of Household and Social Science, Campden Hill Road, London, W. 8.
- 1935 EVANS, A. C., B.Sc., D.I.C., Rothamsted Experimental Station, Harpenden, Herts.
- 1927 EVERETT, J., B.A., Canterton Cottage, Lyndhurst, Hants.
- 1920 FAHMY, T., D.Sc., Mycological Division, Plant Protection Section, Ministry of Agriculture, Giza, Cairo, Egypt.
- 1920 FENTON, E. WYLLIE, M.A., B.Sc., F.R.E.S., Biological Department, Edinburgh, and East of Scotland College of Agriculture, 13, George Square, Edinburgh.
- 1929 FINDLAY, W. P. K., D.Sc., A.R.C.S., D.I.C., Forest Products Research Laboratory, Princes Risborough, Bucks.
- 1935 FISH, S., Department of Agriculture for Victoria, Public Offices, Melbourne, Victoria, Australia.
- 1919 FISHER, K., The School, Oundle, Northampton.
- 1923 FISHER, R. C., B.Sc., Ph.D., Forest Products Research Laboratory, Princes Risborough, Bucks. (Council, 1934-)
- 1931 FOISTER, C. E., B.A., Ph.D., Royal Botanic Garden, Edinburgh.
- 1920 FOX-WILSON, G., N.D.Hort., F.L.S., F.R.E.S., R.H.S. Laboratories, Wisley, Ripley, Surrey. (General and Zoological Secretary, 1935- ; Zoological Secretary, 1933-1934; Council, 1927-1929.)
- 1913 FRYER, J. C. F., O.B.E., M.A., F.R.E.S., Pathological Laboratory, Milton Road, Harpenden, Herts. (President, 1926-1927; Treasurer, 1914-1920; Council, 1921, 1924-1925.)
- 1933 GADD, C. H., D.Sc., Tea Research Institute, St Coombs, Talawakelle, Ceylon.
- 1930 GALLOWAY, L. D., M.A., Imperial Agricultural Research Institute, Pusa, India.
- 1914 GARDINER, Prof. J. S., M.A., F.R.S., Bredon House, Selwyn Gardens, Cambridge. (Vice-President, 1917-1918.)

- 1927 GIBSON, W. H., O.B.E., D.Sc., F.I.C., F.Inst.P., Director, The Linen Industry Research Association, The Research Institute, Lambeg, Co. Antrim, Northern Ireland.
- 1920 GIMMINGHAM, C. T., O.B.E., F.I.C., F.R.E.S., Pathological Laboratory, Milton Road, Harpenden, Herts. (Zoological Editor, 1934- ; Council, 1929-1932.)
- 1920 GLYNNE, Miss M. D., M.Sc., F.L.S., Rothamsted Experimental Station, Harpenden, Herts.
- 1920 GOODEY, T., D.Sc., Institute of Agricultural Parasitology, Winches Farm, Hatfield Road, St Albans. (President, 1934-1935; Council, 1923-1929.)
- 1922 GOODWIN, W., M.Sc., Ph.D., S.E. Agricultural College, Wye, Kent.
- 1908 GOUGH, G. G., B.Sc., 45, Poplar Avenue, Edgbaston, Birmingham.
- 1929 GRAINGER, J., Ph.D., Tolson Memorial Museum, Ravensknowle, Huddersfield.
- 1921 GRAY, Prof. P. H. H., M.A., Macdonald College, Quebec, Canada.
- 1929 GREEN, D. E., M.Sc., R.H.S. Laboratories, Wisley, Ripley, Surrey.
- 1935 GREEN, T. L., Dept. of Zoology, The University, Bristol.
- 1933 GREGOR, J. W., Ph.D., Scottish Plant Breeding Station, Craig's House, Corstorphine, Edinburgh.
- 1934 GRIEVE, B. J., M.Sc., Ph.D., D.I.C., Botany School, University of Melbourne, Victoria, Australia.
- 1921 GRUBB, N. H., M.Sc., East Malling Research Station, East Malling, Kent.
- 1934 GÜSSOW, H. T., F.L.S., F.R.M.S., Dominion Botanist, Central Experimental Farm, Ottawa, Canada.
- 1920 GWYNNE-VAUGHAN, Prof. Dame HELEN, D.B.E., D.Sc., LL.D., F.L.S., Birkbeck College, Chancery Lane, London, E.C. 4.
- 1920 HALKET, Miss A. C., B.Sc., Bedford College, Regent's Park, London, N.W.
- 1930 HALL, W. J., D.Sc., A.R.C.S., F.R.E.S., Director, Citrus Experimental Station, P.O. Mazoe, S. Rhodesia, Africa.
- 1924 HARRIS, R. V., A.R.C.S., B.Sc., East Malling Research Station, East Malling, Kent.
- 1927 HATTON, R. G., C.B.E., M.A., Director, East Malling Research Station, East Malling, Kent. (Council, 1928.)
- 1935 HERFORD, Miss G. M., B.Sc., 1, Frognaal Gardens, Hampstead, N.W. 3.
- 1928 HERFORD, G. V. B., B.A., Biological Field Station, Slough, Bucks.
- 1933 HEY, G. L., M.A., c/o The Murphy Chemical Co., Wheathampstead, Herts.
- 1920 HILEY, W. E., M.A., F.L.S., Dartington Hall Ltd., Totnes, Devon. (Council, 1923-1924, 1929-1930).
- 1920 HILL, Sir A. W., K.C.M.G., M.A., Sc.D., F.R.S., F.L.S., Director, Royal Botanic Gardens, Kew, Surrey.
- 1920 HISCOX, Miss E. R., B.Sc., National Institute for Research in Dairying, Shinfield, Nr. Reading.
- 1933 HOBBY, B. M., B.A., The Hope Department, University Museum, Oxford.
- 1924 HOCKEY, J. F., B.S.A., Pathologist in Charge, Plant Pathology Laboratory, Kentville, Nova Scotia, Canada.
- 1931 HOGGAN, Miss I. A., B.A., M.Sc., Ph.D., Horticulture Building, University of Wisconsin, Madison, Wisconsin, U.S.A.
- 1920 HOLDEN, Prof. H. S., D.Sc., F.R.S.E., F.L.S., Department of Biology, University Park, Nottingham.

- 1919 HORNE, A. S., D.Sc., F.L.S., F.G.S., Botany School, Imperial College of Science, London, S.W. 7.
- 1920 HORTON, E., B.Sc., F.I.C., 10, Crieff Road, Wandsworth Common, London, S.W. 18.
- 1927 HOWES, F. N., M.Sc., Royal Botanic Gardens, Kew, Surrey. (Council, 1930-1931.)
- 1928 HUGHES, A. W. McKENNY, D.I.C., Natural History Museum, S. Kensington, London, S.W. 7.
- Orig. IMMS, A. D., M.A., D.Sc., F.R.S., F.L.S., F.R.E.S., Zoological Laboratory, The Museums, Cambridge. (President, 1930-1931; Vice-President, 1924-1927; Treasurer, 1921-1931; Council, 1914-1920; 1933-1935.)
- 1918 JACKSON, Miss D. J., F.L.S., F.R.E.S., North Cliff, St Andrews, Fife, Scotland.
- 1927 JACOBS, S. E., Ph.D., Bacteriology Department, Imperial College of Science, London, S.W. 7.
- 1927 JAMES, H. C., D.Sc., Zoological Laboratory, The Museums, Cambridge.
- 1927 JARY, S. G., B.A., S.E. Agricultural College, Wye, Kent. (Council, 1934-)
- 1934 JOHNSON, C. G., B.Sc., London School of Tropical Medicine and Hygiene, Gower Street, London.
- 1931 JONES, A. P., M.Sc., Department of Agriculture, The University, Leeds.
- 1934 JONES, E. PARRY, B.Sc., Ph.D., A.I.C.T.A., F.R.E.S., Citrus Experimental Station, P.O. Mazoe, S. Rhodesia, Africa.
- 1927 JONES, G. H., M.A., Plant Protection Section, Ministry of Agriculture, Cairo, Egypt.
- 1927 JOSEPH, E. G., B.Sc., 23, Clanricarde Gardens, London, W. 2.
- 1919 KANNAN, KUNHY, M.A., F.R.E.S., Assistant Entomologist, Governemet of Mysore, Bangalore, S. India.
- 1935 KEARNS, H. G. H., B.Sc., Ph.D., The Research Station, Long Ashton, Bristol.
- 1915 KEEBLE, Sir FREDERICK, C.B.E., M.A., Sc.D., F.R.S., Hammels, Boars Hill, Oxford. (Council, 1920.)
- 1932 KERR, H. W., Ph.D., M.Sc., Director, Bureau of Sugar Experiment Station, Brisbane, Australia.
- 1920 KIDD, F., M.A., D.Sc., Director Low Temperature Research Station, Downing Street, Cambridge.
- 1907 KING, H. H., F.L.S., F.R.E.S., Government Entomologist, Wellcome Tropical Research Laboratories, Khartoum, Sudan.
- 1907 KING, Prof. L. A. L., M.A., West of Scotland Agricultural College, 6, Blythwood Square, Glasgow.
- 1926 KINGSTON, H. T., 299, Hertford Road, Waltham Cross, Herts.
- 1933 KRAMER, L. M. J., M.A., Ph.D., Gresham's School, Holt, Norfolk.
- 1921 LACEY, Miss M. S., M.Sc., Botanical Department, Imperial College of Science, London, S.W. 7.
- 1921 LAURIE, Prof. R. D., M.A., F.Z.S., Department of Zoology, University College of North Wales, Aberystwyth.
- 1928 LAWRENCE, E., N.D.A., Department of Agriculture, Zomba, Nyasaland, British East Africa.
- 1933 LEACH, R., B.A., A.I.C.T.A., 7, Wellington Square, Cheltenham.
- 1934 LEAN, O. B., B.Sc., D.I.C., Imperial Chemical House, Millbank, S.W. 1.
- 1926 LE PELLEY, R. H., Ph.D., Department of Agriculture, Nairobi, Kenya, Africa.

- 1920 LLOYD, LLEWELLYN, D.Sc., Zoological Department, The University, Leeds.
- 1931 McCLEAN, A. P. D., M.Sc., Natal Herbarium, Berea, Durban, Natal, Union of S. Africa.
- 1914 McCLELLAN, F. C., C.B.E., M.R.A.C., F.L.S., Director of Agriculture, Zanzibar.
- Orig. MACDOUGALL*, Prof. R. S., M.A., D.Sc., F.R.S.E., F.R.E.S., Ivy Lodge, Gullane, E. Lothian, Scotland. (Vice-President, 1914-1919; Council, 1908-1913.)
- 1929 MACGILL, Miss E. I., D.Sc., Linkfield, Hawthorn Road, Denton, Manchester.
- 1927 McLENNAN, Miss ETHEL, D.Sc., Botanical Department, The University, Melbourne, Australia.
- 1925 MACLEOD, D. J., M.A., Officer in Charge, Dominion Plant Pathological Laboratory, Fredericton, New Brunswick.
- 1909 MANGAN, Prof. J., M.A., 3, Ceiriog Close, Penarth, Glamorgan, S. Wales.
- 1920 MANGHAM, Prof. S., M.A., University College, Southampton.
- 1917 MANN, H. H., D.Sc., F.L.S., Woburn Experimental Station, Aspley Guise, Bedfordshire.
- 1914 MARSHALL, Sir GUY, A. K., D.Sc., C.M.G., F.R.S., F.Z.S., F.R.E.S., Director, Imperial Institute of Entomology, Natural History Museum, London, S.W. 7. (Vice-President, 1920, 1922; Council, 1917-1922.)
- 1934 MARSHALL, J., B.Sc., A.R.C.S., A.I.C.T.A., Empire Cotton Growing Corporation, 2, Wood Street, Millbank, S.W. 1.
- 1930 MARTIN, H., D.Sc., A.R.C.S., Research Station, Long Ashton, Bristol.
- 1922 MASON, E. W., M.A., M.Sc., Imperial Mycological Institute, Kew, Surrey.
- 1920 MASON, F.A., F.R.M.S., 29, Frankland Terrace, Leopold Street, Leeds.
- 1933 MASON, Miss M., B.Sc. (Hort.), Middlesex Education Committee, 10, Great George Street, London, S.W. 1.
- 1927 MASSEE, A. M., D.Sc., F.R.E.S., East Malling Research Station, East Malling, Kent. (Council, 1932-1934.)
- 1920 MATTICK, A. T. R., B.Sc., Ph.D., National Institute for Research in Dairying, Shinfield, Nr. Reading.
- 1933 MEIKLEJOHN, Miss J., Ph.D., Rothamsted Experimental Station, Harpenden, Herts.
- 1932 METCALFE, Miss M. E., B.Sc., Ph.D., 20, Llantwit Road, Treforest, Glamorgan-shire.
- 1923 MILES, H. W., M.Sc., Ph.D., Department of Agricultural Entomology, Victoria University, Manchester.
- 1921 MILLARD, W. A., D.Sc., Department of Agriculture, The University, Leeds.
- 1932 MISRA, A. B., D.Sc., Ph.D., F.Z.A., F.R.E.S., Department of Zoology, Benares Hindu University, India.
- 1932 MOORE, Miss E. S., B.Sc., Ph.D., Tobacco Research Laboratory, Balfour, C.P., S. Africa.
- 1928 MOORE, W. C., M.A., Plant Pathological Laboratory, Milton Road, Harpenden, Herts.
- 1922 MORLAND, D. M. T., M.A., Rothamsted Experimental Station, Harpenden, Herts.
- 1920 MORRIS, H. M., M.Sc., Agricultural Department, Nicosia, Cyprus.

- 1920 MOSLEY, F. O., F.L.S., Pathology Laboratory, Messrs Lowe and Shawyer, Uxbridge, Middlesex.
- 1925 MUMFORD, E. PHILPOTT, B.Sc., "Old Romney", Beaconsfield.
- 1914 MUNRO, Prof. J. W., M.A., D.Sc., Zoology Department, Imperial College of Science, London, S.W. 7. (Vice-President, 1933; General and Zoological Secretary, 1928-1932; Zoological Editor, 1933; Council, 1922-1924, 1927.)
- 1919 MURPHY, A. J., Technical Chemist, The Murphy Chemical Co., Wheathampstead, Herts.
- 1920 MURPHY, Prof. P. A., D.Sc., B.A., Albert Agricultural College, Glasnevin, Dublin, Irish Free State.
- 1925 MUSKETT, A. E., B.Sc., A.R.C.S., The Queen's University, Belfast, N. Ireland.
- 1928 NEL, R. I., B.Sc., M.Sc., Naga Hoeta Estate, Pematang Siantar, Sumatra (East Coast).
- 1928 NEWTON, H. C. F., B.Sc., A.R.C.S., D.I.C., Harper Adams Agricultural College, Newport, Salop.
- 1930 NORMAN, A. G., D.Sc., F.I.C., Rothamsted Experimental Station, Harpenden, Herts.
- 1920 NOWELL, W., D.I.C., F.L.S., Director, East African Agricultural Research Station, Amani, Tanga, Tanganyika, E. Africa.
- 1928 NUTMAN, F. J., B.Sc., A.R.C.S., East African Agricultural Research Station, Amani, Tanga, Tanganyika, E. Africa.
- 1923 OGILVIE, L., M.A., M.Sc., Research Station, Long Ashton, Bristol.
- 1925 OLDHAM, J. N., B.Sc., Ph.D., Research Institute of Animal Pathology, Royal Veterinary College, Camden Town, London, N.W. 1. (Council, 1932-1934.)
- 1919 PAINE, Prof. S. G., D.Sc., F.I.C., Imperial College of Science, London, S.W. 7. (General and Botanical Secretary, 1923-1926; Council, 1927.)
- 1930 PARKER, W. H., M.C., M.A., Director, National Institute of Agricultural Botany, Huntingdon Road, Cambridge.
- 1928 PARKIN, E. A., B.Sc., Forest Products Research Laboratory, Princes Risborough, Bucks.
- 1914 PETHERBRIDGE, F. R., M.A., School of Agriculture, Cambridge.
- Orig. PETHYBRIDGE, G. H., M.A., M.R.I.A., Ph.D., B.Sc., Pathological Laboratory, Milton Road, Harpenden, Herts. (Vice-President, 1927; Council, 1912-1913, 1925-1927.)
- 1928 PICKLES, A., B.Sc., Imperial College of Tropical Agriculture, Trinidad, B.W. Indies.
- 1915 PORTER*, A., D.Sc., Department of Zoology, McGill University, Montreal, Canada.
- 1934 POTTER, C., B.Sc., Entomology Department, Imperial College of Science, London, S.W. 7.
- 1928 POTTER, Mrs E. M., B.Sc., Entomology Department, Imperial College of Science, London, S.W. 7.
- 1907 POULTON*, Prof. Sir EDWARD B., M.A., D.Sc., LL.D., F.R.S., Wykeham House, Banbury Road, Oxford. (President, 1922-1923; Vice-President, 1903-1909, 1924-1925.)

- 1919 PRAIN*, Sir DAVID, Lt.-Col., C.M.G., C.I.E., M.A., M.B., F.R.S., LL.D., F.R.S.E., V.M.H., The Well Farm, Warlingham, Surrey. (President, 1920-1921; Vice-President, 1924-1927.)
- 1934 PRATT, A., B.Sc., Bell-Baxter School, Cupar, Fife.
- 1923 PRESTON, N. C., B.Sc., Harper Adams Agricultural College, Newport, Salop.
- 1928 RAMSBOTTOM, J., O.B.E., M.A., F.L.S., British Museum (Natural History), Cromwell Road, London, S.W. 7.
- 1931 REICHERT, I., Ph.D., Chief Plant Pathologist, P.O. Box 15, Rehoboth, Palestine.
- 1921 RICHARDS, E. H., B.Sc., F.I.C., Rothamsted Experimental Station, Harpenden, Herts.
- 1928 RICHARDS, O. W., M.A., D.Sc., 29a, Edith Road, London, W. 14.
- 1921 ROACH, W. A., B.Sc., A.R.C.S., D.I.C., A.I.C., East Malling Research Station, East Malling, Kent. (Council, 1935-)
- 1914 ROBERTS, A. W. RYMER, M.A., F.R.E.S., Molteno Institute for Research in Parasitology, Cambridge.
- 1923 ROBINSON, D. H., B.Sc., Harper Adams Agricultural College, Newport, Salop.
- 1918 ROBSON, R., Institute of Agriculture, Chelmsford.
- 1920 ROEBUCK, A., N.D.A., Midland Agricultural College, Sutton Bonnington, Loughborough, Derby.
- 1929 ROGERS, W. S., B.A., East Malling Research Station, East Malling, Kent.
- 1919 RUSSELL, Sir E. JOHN, D.Sc., F.R.S., Director, Rothamsted Experimental Station, Harpenden, Herts. (Vice-President, 1923; Council, 1921-1923.)
- 1932 RUSSELL, T. A., B.Sc., A.R.C.S., Department of Agriculture, Paget East, Bermuda.
- 1929 SALAMAN, R. N., M.D., F.R.S., Director, Potato Virus Research Station, School of Agriculture, Cambridge.
- 1914 SALMON, Prof. E. S., F.L.S., S.E. Agricultural College, Wye, Kent. (Council, 1916, 1924-1926.)
- 1923 SAMUEL, G., B.Sc., Rothamsted Experimental Station, Harpenden, Herts. (Council, 1935-)
- 1921 SARGENT, R. H., Technical College, Darlington.
- 1933 SCHIMMER, Miss F. C., M.Sc., A.R.C.S., D.I.C., c/o Mrs Church, 13 Old Sq., Lincoln's Inn, London, W.C. 2.
- 1919 SEARLE, G. O., B.Sc., Linen Industry Research Association, The Research Institute, Lambeg, Co. Antrim, N. Ireland.
- 1932 SHARGA, U. S., M.Sc., Ph.D., Agricultural Dept., Etawah, U.P., India.
- 1932 SHEFFIELD, Miss F. M. L., Ph.D., F.L.S., Rothamsted Experimental Station, Harpenden, Herts.
- 1920 SMALL, Prof. J., D.Sc., F.L.S., Queen's University, Belfast, N. Ireland.
- 1928 SMALL, T., M.Sc., A.R.C.S., The States Experimental Farm, Trinity, Jersey.
- 1920 SMITH, E. HOLMES, B.Sc., Botany School, Victoria University, Manchester.
- 1919 SMITH, J. HENDERSON, M.B., Ch.B., B.A., Department of Plant Pathology, Rothamsted Experimental Station, Harpenden, Herts. (Treasurer, 1932-).
- 1920 SMITH, KENNETH M., D.Sc., Ph.D., Potato Virus Research Station, School of Agriculture, Cambridge.

- 1927 SMITH, Prof. N. J. G., M.A., B.Sc., Ph.D., Rhodes University College, Grahams-town (Cape), S. Africa.
- 1913 SOUTH, F. W., M.A., Agricultural Department, Kuala Lumpur, Federated Malay States.
- 1919 SPEYER, E. R., M.A., Experimental and Research Station, Cheshunt, Herts. (Council, 1931-1933.)
- 1920 SPINKS, G. T., M.A., Research Station, Long Ashton, Bristol.
- 1920 STAPLEDON, Prof. R. G., C.B.E., M.A., Director, Imperial Bureau of Plant Genetics, Agricultural Buildings, Alexandra Road, Aberystwyth.
- 1933 STEER, W., Danelough, London Road, Leybourne, West Malling, Kent.
- 1925 STELL, F., Department of Agriculture, Port of Spain, Trinidad, British W. Indies.
- 1921 STENTON, R., F.R.E.S., 22, Milton Road, Harpenden, Herts. (Council, 1928-1930.)
- 1922 STIRRUP, H. H., M.Sc., Midland Agricultural College, Sutton Bonnington, Loughborough, Derby.
- 1919 STONE, H., "Long Reach", Chesterton Fen, Nr. Cambridge.
- 1926 STOREY, H. H., M.A., Ph.D., East African Agricultural Research Station, Amani, Tanga, Tanganyika, E. Africa.
- 1927 STOUGHTON, Prof. R. H., D.Sc., A.R.C.S., The University, Reading, Berks. (General and Botanical Secretary, 1933-1934; Botanical Secretary, 1935-)
- 1933 SUTTON, M. A. F., F.L.S., Sutton and Sons, Ltd., Reading, Berks.
- 1919 TABOR, Prof. R. J., B.Sc., Botany School, Imperial College of Science, London, S.W. 7.
- 1935 TARR, H. L. A., Ph.D., Rothamsted Experimental Station, Harpenden, Herts.
- 1921 TATTERSFIELD, F., D.Sc., F.I.C., Rothamsted Experimental Station, Harpenden, Herts.
- 1914 TAYLOR, H. V., O.B.E., B.Sc., A.R.C.S., Commissioner for Horticulture, Ministry of Agriculture and Fisheries, 10, Whitehall Place, London, S.W. 1. (Council, 1927-1928.)
- 1930 TETLEY, Miss H. U., Quarry Garth, Windermere, Westmorland.
- 1927 THAYSEN, A. C., Ph.D., 3, Cumberland Court, 83, West Hill, S.W. 15. (Council, 1933-1935.)
- 1931 THOMAS, I., M.Sc., School of Agriculture, Cambridge.
- 1933 THOMSON, J. R., B.Sc., The University, Reading, Berks.
- 1919 THORNTON, H. G., B.A., D.Sc., Rothamsted Experimental Station, Harpenden, Herts.
- 1933 THOROLD, C. A., B.Sc., Department of Agriculture, Nairobi, Kenya.
- 1934 THORPE, W. H., M.A., Ph.D., Zoological Laboratory, The Museums, Cambridge.
- 1927 TINCKER, M. A. H., M.A., D.Sc., R.H.S. Laboratories, Wisley, Ripley, Surrey. (Council, 1930-1932.)
- 1930 TOMMERUP, ERIC C., B.Sc., c/o Mrs A. G. Parry, 4, John Street, Ashfield, Sydney, Australia.
- 1919 TROW, Principal A. H., D.Sc., F.L.S., 9, Clive Crescent, Penarth, Glamorgan.
- 1927 TURNER, W. H., B.Sc., Technical Department, Geo. Monro, Ltd., Waltham Cross, Herts.

- 1913 URICH, F. W., Imperial College of Tropical Agriculture, Trinidad, British West Indies.
- 1915 VAN DER BYL, Prof. P. A., M.A., D.Sc., F.L.S., University of Stellenbosch, Stellenbosch, Union of S. Africa.
- 1921 VOELCKER, J. A., M.A., B.Sc., Ph.D., F.I.C., Stuart House, 1, Tudor Street, London, E.C. 4.
- 1929 VYVYAN, M. C., M.A., East Malling Research Station, East Malling, Kent.
- 1920 WAKEFIELD, Miss E. M., M.A., F.L.S., Royal Botanic Gardens, Kew, Surrey.
- 1929 WAKELY, C. T. N., B.Sc., Africa House, Kingsway, London, W.C. 2.
- 1932 WALDIE, J. S. L., B.Sc., N.D.A., The University, Reading, Berks.
- 1923 WALKDEN, H., The Raft, Derbyshire Road, Sale, Cheshire.
- 1931 WALLACE, E. R., B.A., Agricultural Institute, Kirton, Nr. Boston, Lincs.
- 1928 WALLACE, G. B., B.Sc., Ph.D., Department of Agriculture, Morogoro, Tanganyika Territory, E. Africa.
- 1919 WALLACE, J. C., M.C., Director, Agricultural Institute, Kirton, Nr. Boston, Lincs. Orig. WARBURTON, C., M.A., Yew Garth, Grantchester, Cambridge. (Council, 1905-1913.)
- 1919 WARE, W. M., M.Sc., S.E. Agricultural College, Wye, Kent. (Council, 1930-1933.)
- 1922 WARINGTON, Miss K., M.Sc., Rothamsted Experimental Station, Harpenden, Herts.
- 1930 WATSON, Mrs M. A., Ph.D., Rothamsted Experimental Station, Harpenden, Herts.
- 1920 WATT, A. S., Ph.D., B.A., Forestry Department, Cambridge.
- 1918 WEST, C., D.Sc., A.R.C.S., D.I.C., F.L.S., Low Temperature Research Station, Downing Street, Cambridge. (Council, 1931.)
- 1923 WESTON, W. A. R. DILLON, M.A., Ph.D., School of Agriculture, Cambridge.
- 1921 WHITEHEAD, T., D.Sc., A.R.C.S., University College of North Wales, Memorial Buildings, Bangor.
- 1912 WILLIAMS, C. B., Sc.D., Rothamsted Experimental Station, Harpenden, Herts. (Vice-President, 1934-1935; Council, 1933-1935.)
- 1930 WILLIAMS, P. H., B.Sc., Experimental and Research Station, Cheshunt, Herts. (Council, 1934-)
- 1919 WILLIS, J. C., M.A., Sc.D., F.R.S., F.L.S., 8, Cavendish Avenue, Cambridge.
- 1923 WILSON, Miss A. P., A.R.C.S., 116, Fellows Road, Hampstead, London, N.W.
- 1933 WILSON, A. R., B.Sc., c/o Dr Gregor, North Clermiston House, Cramond Bridge, West Lothian, Scotland.
- 1914 WILSON, M., D.Sc., A.R.C.S., Royal Botanic Garden, Edinburgh.
- 1931 WILSON, Prof. S. E., Ph.D., Royal Veterinary College, Camden Town, London, N.W. 1.
- 1921 WILTSHIRE, S. P., M.A., D.Sc., Imperial Mycological Institute, Kew, Surrey. (Council, 1926-1929; 1935-)
- 1932 WOOD, J., A.R.C.S., D.I.C., Agricultural Institute, Kirton, Nr. Boston, Lincs.
- 1926 WOODWARD, R. C., M.A., Imperial Chemical Industries, Pest Control Section, Millbank, S.W. 1. (Council, 1931-1932.)
- 1914 WORMALD, H., D.Sc., A.R.C.S., East Malling Research Station, East Malling, Kent.
- 1920 WORTLEY, E. J., F.I.C., M.B.E., F.C.S., Director of Agriculture, St Anns, Port of Spain, Trinidad, British West Indies.
- 1930 YOUNG, W. H., The Nurseries, Kings Road, Lancing, Sussex.

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